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MORPHOLOGICAL NOTES TOGETHER WITH SOME ULTRAFILTRATION EXPERIMENTS ON THE CROWN-GALL PATHOGENE, BACTERIUM TUMEFACIENS

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(WITH PLATES 24 AND 25)

The recent work of Levine (**8**) on morphological changes in *Bacterium tumefaciens* is of considerable interest from a scientific as well as from a practical standpoint. Levine reports that smears from a three-months-old culture of this organism on bean agar, when stained with Loeffler's methylene blue, show an amorphous mass of jelly-like substance with occasional deeply stained minute spherical bodies. He reports that smears from young cultures, representing transfers from the old bean culture, show long rods which not infrequently present a beaded appearance. Likewise, he finds an abundance of small lenticular bodies staining heavily at both ends, while the central portions remain unstained. These bodies he takes to be spores which, when transferred to fresh media, germinate and give rise to a rod-like body not unlike *Bacterium tumefaciens* in size. Whether these bodies are to be regarded as comparable to the "minute spherical bodies" of the old bean culture is not stated, although it is noted that in old cultures the spores appear to lose their ability to stain and may be recognized as the bodies most abundant in the smear.

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The writer's first interest in the morphology of the crown-gall pathogene was from the point of view of staining its flagella, for, in spite of the enormous mass of literature which has accumulated on this organism and on the disease it produces, there is not a single clear photograph in any publication known to the writer showing the flagella. True it is that Smith, Brown, and Townsend (**12**, p. 107) give drawings of the organism showing flagella, and Smith (**13**) elsewhere presents a rather hazy photograph, but in view of the large numbers of excellent microphotographs of various sorts that have been published on the crown-gall disease, it is rather surprising to find such few photographs of the organism. In all probability, this may be explained by the fact that flagella of this organism are not easy to stain.

It must be noted that while the writer has been studying *B. tumefaciens* for upwards of two years, interpretation of various forms is still questionable to him. I shall simply state what these forms are and suggest certain possibilities as to their function. By means of the flagella stain which the writer has developed, excellent preparations have been obtained, showing not only the flagella (see upper part of PLATE 24), but also certain interesting features of the bodies themselves. That these have been clearly seen by this method is not surprising, inasmuch as it entirely eliminates heating the film in order to fix it to the slide, and hence eliminates a process (roasting or broiling would be a proper name for it) which is almost certain to destroy any minute structures within the body, as well as to render any irregularities in form uncertain of detection.

In young, vigorously growing cultures of *Bacterium tumefaciens*, representing transfers of cultures kindly supplied by Dr. A. J. Ricker of the University of Wisconsin, stained as above mentioned, the variation in size and shape of the rods is very conspicuous. (It must be emphasized that old cultures, or cultures in which the organisms make little or no growth, are not being considered at this time.) Vigorously growing culture as here used means one in which a very conspicuous growth along the whole length of a slant of nutrient agar kept at 28° C. is obtained in 18 to 24 hours. A large number of measurements of bacteria coming from such cultures and from cultures up to four days old,

stained by the writer's flagella-staining method, show the following variations in size: 0.6 to 3.6 by 0.3 to 1.0 μ. As to relative frequency, measurements less than 1.0 μ for the long diameter are comparatively few, while measurements around 3.6 μ are also relatively few. The latter refer to figures in which two potential cells remain united and give no clear indications of being separate individuals, *i.e.* with no clearly distinguishable cross walls and without ends rounded at the point of contact. As to the short diameter, measurements below 0.5 μ are common, indeed just as common as measurements above 0.5 μ. A convincing picture of the diversity of sizes obtainable in *B. tumefaciens* may be obtained from the following measurements of organisms representing young cultures.

0.6 × 0.3 μ	1.0 × 0.3 μ
0.9 × 0.5 μ	1.2 × 0.5 μ
1.0 × 0.6 μ	1.0 × 0.4 μ
0.8 × 0.3 μ	3.5 × 0.8 μ
1.0 × 0.4 μ	1.5 × 0.6 μ
1.2 × 0.7 μ	0.8 × 0.3 μ
1.5 × 0.6 μ	2.4 × 0.9 μ
1.2 × 0.4 μ	3.0 × 0.9 μ
2.4 × 0.8 μ	1.5 × 1.0 μ
1.5 × 0.4 μ	3.6 × 0.8 μ

With such diversity, what significance may be attached to average or mean measurements? Hence the lack of close agreement among different workers on the same organism. Smith, Brown, and Townsend (**12**, p. 106) state that when taken from young agar cultures the limits of size are 1 to 3 by 0.4 to 1.8 μ and that the majority measure 1.2 to 2.5 by 0.5 to 0.8 μ. Walkden's (**16**) sizes of unstained bodies representing young agar cultures are: 1.2 to 1.5 by 0.5 to 0.6 μ, paired rods 2.8 to 3.0 by 0.5 to 0.6 μ. Ricker (**10**) states that the size limits of the organism, growing on agar, isolated by him from a raspberry gall, are 1 to 1.6 by 0.4 to 0.7 μ and that the size of the majority are 1.1 by 0.5 μ. Really these "average" measurements by different workers are in closer agreement than one should expect. They reveal the almost universal attitude of bacteriological workers of looking upon all normal bacteria as capable of showing only one

form and of possessing but one means of reproduction, one in which daughter cells and mother cells are alike except in the long diameter.

Having presented figures showing the diversity of sizes, the next question to be considered is the diversity of forms. From the sizes one may surmise that some of the rods are rather minute, while others are fair sized. But one of the most interesting and characteristic features of the crown-gall organism is the common occurrence of individuals much wider at one end than at the other, giving the appearance of short, stocky clubs. When heated and heavily stained they usually appear homogeneous throughout. Stained by the more delicate, flagella-staining method, they frequently can be seen as plump rods which appear to be distended at one end (see PLATES 24 and 25). This extension comprises the narrow portion of the club and internally is associated with the presence of heavily staining material, reaching from the main body of the rod into the narrow portion (see PLATE 25).

In some of the figures there is a more or less definite suggestion of a nuclear phenomenon occurring at the point of extension or elongation and suggesting, at least in gross appearance, the ordinary budding process of yeast cells. Another interpretation of this is that the main body represents a bacterial spore which is in the process of germination. Lastly, there is the possibility that these extensions represent some abnormal or senility process, in which the old rod is disintegrating, pushing out its contents prior to its death and prior to its final form as a ghost cell. The last explanation need not be entirely overlooked (ghost cells are occasionally observed in young, rapidly growing cultures). That they are not mere artifacts due to the process of mordanting and staining may readily be proved by observing living cultures in hanging drops. When this is done, clubs looking very much like those in stained preparations may be commonly observed, and occasionally there is a strong suggestion in this living material of internal structures comparable to those seen in stained preparations. In addition to the extension from one end of the rod, rods occasionally appear with extensions from both ends (see PLATE 25), and in these figures there is also to be noted an internal,

heavily staining mass of material at each end either in the act of pushing out from the end of the rod into the bud or separated into two masses lying close together, one being in the end of the main body and the other in the bud-like extension. These buds vary considerably in size, sometimes being but a mere fraction of the rod and grading into bodies almost as long as the mother cell, and occasionally as wide. Very often they are seen to possess heavily staining bodies at each pole.

As to the internal structure of rods which possess no buds or extensions at the poles, there is to be seen, both in living material and in stained preparations, marked difference in different parts. Stained, they are very frequently seen as more or less lens-shaped bodies, heavily stained at both ends and aside from the wall, which can be definitely seen in this organism (see PLATES 24 and 25), appear perfectly colorless toward the center. These deeply colored bodies are undoubtedly to be interpreted as polar bodies which have been noted in many non-spore-forming bacteria, and whose functions are quite debatable. It is quite probable that it is these bipolar, lens-shaped organisms which Levine takes to be spores. When seen unstained, in hanging drop cultures, the ends as noted above appear different, being more refractive than the centers, but when placed alongside of true bacterial spores, as I had occasion to observe in a mixed culture of *B. tumefaciens* with some large, unknown, peritrichous, spore-forming rod, then the difference between the true spore cell and the cells of *B. tumefaciens* with refractive ends was very marked indeed. The very glistening, round, highly refractive spores and the very slightly refractive and non-glistening ends of the crown-gall pathogene can hardly be considered the same. Spore staining methods used by the writer point to a similar conclusion.

The question of spore formation in this organism is apt to be considered a very important one. If this is a spore former, what is the likelihood of control by washing seedlings and scions for 10 minutes in a 0.25 per cent hydroxymercurichlorophenol solution, and dipping the grafts in this same strength of solution, as Waite and Siegler (**15**) recommended?[1] In the older literature, and

[1] Spores of the tetanus bacillus are killed only after three hours in a 1 to 1000 mercuric chloride solution, and spores of the anthrax bacillus in two hours in

occasionally in more recent articles dealing with phytopathogenic bacteria, there appear statements to the effect that spores appear in the cultures. Thus Burrill (**3**) described spores for the organism causing sorghum blight, and Manns (**9**) found spores in old cultures of *Pseudomonas Avenae*, an organism causing the blade blight of oats. Others might be mentioned, but these two well-known diseases and their causal agents are good examples of errors which have crept in, mostly in the older literature. (For a good preliminary description of the sorghum pathogene, see Charlotte Elliott (**5**) and for the oat organism see (**4**) by the same author.) Indeed, there is not a single, authenticated, bacterial plant pathogene known to the writer which is known to produce spores. While it is never safe to generalize in such matters, the large number of phytopathogenic bacteria known at present to be non-spore formers makes it unsafe to accept statements to the contrary, unless accompanied by a large amount of unimpeachable evidence. Are there any bacteria, saprophytes or parasites, known to produce spores which are not large-sized organisms? If not (**2**), what are the possibilities of comparatively short or small-sized rods, characteristic of most and perhaps all plant pathogens, of producing spores? Coupled with this, it will be recalled that all bacterial phytopathogens form a rather compact group, in many respects very closely related, not a single coccus type being definitely known to be a plant pathogene. (In the older literature these are of course recorded, Burrill's *Micrococcus amylovorus*, Prillieux' *Micrococcus* associated with discolored wheat kernels, etc.)

There is other convincing evidence that spores are not formed in *Bacterium tumefaciens*. The writer has quite often failed to obtain transfers from cultures several months old, the old media being nutrient broth, nutrient agar, and potato glucose agar. Very good evidence can be found in the thermal relationships of this pathogene. Smith and his co-workers (**12**, p. 120) found it to be killed when exposed for 10 minutes in beef broth at about 51° C. and when exposed on agar or broth for seven days at 37 to 37.4° C., they likewise found it to be dead. Walkden (**15**) found

the same strength solution. (The strength is very comparable in killing power to the 0.25 per cent organic mercury solution.)

the thermal death point to be between 44 and 46° C. The writer has obtained similar results. In view of all this evidence, it is safe to conclude that *Bacterium tumefaciens* does not produce spores.

In addition to the ends of the rod and extensions from these showing deeply staining bodies, there is often to be observed a more or less roundish body, sometimes seen as a heavy band, near the centers of medium- or large-sized rods (see upper figure, PLATE 25). Such central bodies have mostly, if not always, been found in rods which show only one polar body. Not infrequently, instead of a solid band, there appear two small bodies opposite each other and also in the equatorial region of the rod (see PLATE 25, middle, right-hand figure). In rods measuring 3 μ or more in length, there is to be noted at times two regions, each representing two particles arranged opposite each other, in various ways suggesting mitotic figures (see large rod in PLATE 25, middle, right-hand figure). However, it would be unwise to insist that these represent true spindles, for the bodies are so tiny that even with the highest magnifications their forms and the connection between them are not clearly discernible. In addition to the one or two bands or pairs of bodies, there are various other figures which suggest a nuclear phenomenon prior to cell division (see upper figure, PLATE 25). Very frequently a cleavage line can be seen separating two heavily stained, closely joined ends and associated with this there is a greater or lesser amount of invagination at this point. A suggested interpretation for this is as follows.

There is a regular nuclear division followed by a cell division in which there is no true cell plate formation, but rather a pushing in or cleavage plane formed, beginning at the outer walls and finally extending clear through the width of the rod. Such a process is of course well known in many fungi, and perhaps in other plants. But, regardless of the true interpretation of these internal bodies, and the writer is certainly far from settled on any one explanation, their presence at the point of division throws considerable burden on those who look upon bacteria as devoid of anything suggesting a true nucleus. The writer has already questioned this assumption in an address delivered before the

Mycological Section of the American Botanical Society (American Assoc. Adv. Sci. meeting at Cincinnati, December, 1924), and expects to discuss this more fully in a later article. I am not absolutely certain that only in rods with a single polar body do such division figures appear, but if this is true can it mean that one or both of the polar particles represent nuclei, one of which moves to the center of the body when it is ready to divide?

ULTRAFILTRATION EXPERIMENTS

The very small-sized rods and buds or extensions derived from larger rods, as previously described, brought up the question of the possibility of filtering these through bacteriological filters. If these minute bodies are alive, could they not pass through a porcelain filter and produce typical growth after such passage? To test this out, a steam-sterilized Berkefeld V filter was used. A 48-hour culture growing in an Erlenmeyer flask containing 350 cc. of nutrient broth, kept at 29° C., was poured into the mantel and a vacuum of about 630 to 711 mm. of mercury utilized for around 20 minutes. The filtrate was then carefully transferred in various quantities by means of sterilized pipettes, the transferring being done in a small, completely enclosed chamber, which had previously been heavily steamed. Transfers were made into tubes of nutrient broth and into petri dishes, to which melted, nutrient agar was added. Contaminations in the form of large rods, in rather rapidly growing, irregular-margined colonies were noted on practically all of the plates and in some of the broth tubes. But thanks to the careful work of Smith and his associates, the plates and tubes, all apparently showing no growth, typical of *B. tumefaciens*, were kept under close observation for over a week. All the plate colonies which appeared within four days after transfer were recognized as contaminations. Beginning with the fourth day, very small, round, glistening, whitish, almost translucent, colonies were noted in small numbers on some of the plates. They grew slowly and remained small, not more than 2 to 4 mm. up to the eighth day. When transferred to nutrient agar slants they made typical growth, though not with the wrinkled appearance which is obtainable in cultures representing numerous previous transfers from various slants.

As there is a possibility of flaws even with the best of filters, the same filter was utilized for filtering a culture of the fire-blight pathogene, *Bacillus amylovorus*. The culture used represents an isolation made by the writer in May, 1925, from a blighted apple and has been previously reported on by the writer (**11**). As the mature rods of this organism are smaller than those of the crown-gall pathogene, and as no tiny bodies comparable to those found in the latter were observed, it would appear that the use of *B. amylovorus* for ultrafiltration constitutes a very rigid check. Transfers from the filtrate, made in the same way and in the same quantities, were entirely free from *B. amylovorus*.

In addition to transferring part of the filtrate of the crown-gall organism as previously described, the remaining filtered broth was then centrifuged at about 1200 revolutions per minute for 20 minutes, and all but the liquid closest to the bottom of the flasks discarded. This was then examined in hanging drops and in stained preparations. The examinations showed typical short rods, containing the bipolar, heavily staining bodies, in all respects characteristic of *B. tumefaciens*. They were rather few in number, and not a single medium- or large-sized rod was observed. The transfers from the plates on nutrient agar slants were also carefully examined and stained, and the characteristic bud-like processes noted on many of the rods. In all other respects the stained preparations, representing colonies derived from filtered material, were comparable to the typical forms of *B. tumefaciens*.

These filtration experiments, while in a preliminary stage and requiring further work, are of considerable interest not only because they throw some light on the morphology of the crown-gall organism, but also because of their possible application to the work being done on human cancer and that on certain filterable viruses. The recent article by Smith (**14**) calling attention to work of foreign cancer specialists is worthy of mention. He states that certain German investigators, Ferdinand Blumental and his associates, have isolated a schizomycete from human breast cancers, which in pure culture resembles *B. tumefaciens*. With this organism, together with heated cancerous breast serum and diatomaceous earth, they have been able to cause malignant

transplantable tumors in rats and crown-galls in plants. Furthermore, this work has apparently been substantiated by other German workers in another laboratory, Reichert and his coworkers, who have done away with the use of cancer serum as an added irritant and who, using pure cultures of the PM strain of the organism isolated by Blumental and his associates, have been able to produce metastasizing, malignant, transplantable tumors in rats.[2] Along with these findings and apparently in disagreement with them, Smith reports the work of Gye and Barnard of England. The former has been able to cultivate the virus of Rous' chicken sarcoma in test tubes and has obtained numerous infections in chickens by the use of these cultures. In spite of this, the test tube liquid cultures show no observable pellicle, precipitate or clouding, nor is anything visible under the highest powers of the microscope, except when the culture fluid is centrifuged. Then it is that the virus may be found at the bottom of the tubes in a "particulate" form. Gye (**7**) further reports that two factors are necessary for the production of a tumor; one is the virus and the other some labile chemical substance left in the supernatant liquid of centrifugalized cultures, termed the specific factor. He also reports mouse and rat cancers as being transmissible by a cell-free filtrate and by virus cultures. Human tumors were likewise investigated. When cultures from one of these were inoculated in chickens and the specific factor added, tumors indistinguishable from Rous' chicken sarcoma were obtained.

But Barnard's morphological studies (**1**) of the viruses isolated by Gye are of particular interest to the writer. He reports that by the use of special light arrangements attached to a microscope he is able to observe the particles constituting the virus and has concluded that they are comparable to the organisms (also filterable) causing bovine pleuro-pneumonia. The latter show sphaeroids on the sides of which small refractive particles are formed, an arrangement which doubtless suggested yeast-like

[2] This is not the first time that investigators have reported finding an organism in human beings which is referable to *B. tumefaciens* and which is capable of producing crown-gall in plants. About eleven years ago, Friedemann and Magnus (**6**) reported that from the faeces of a sick human they isolated an organism which produced crown-galls in plants.

germination, and hence the pathogene was considered a yeast by one or more former investigators. Usually the sphaeroid disintegrates, while some of the particles develop into small sphaeroids, each of which produces attached particles in turn. Considerable observation on infective cultures has led Barnard to conclude that the same type of phenomenon, but on a smaller scale, is to be observed in the Rous' chicken sarcoma, in a mouse sarcoma, and in human carcinoma.

As the writer has already recorded, *B. tumefaciens* in young vigorously growing cultures produces buds or extensions from the regular rods, often seen as short, plump, lens-shaped bodies, which resemble in various aspects the budding of yeast cells. Is it not a remarkable coincidence that a comparable type of structure should be noted in the crown-gall pathogene and in the virus of various forms of cancer? Also, given a medium which may be better adapted to the growth of these cancer viruses, may not the growths then be heavier and hence more observable? In spite of Dr. Barnard's statement to the contrary, there is that possibility and it can be exemplified in the work of Noguchi on yellow fever, in which a filterable virus is also involved. To the writer the work of Barnard and Gye and that of Blumental and Reichert is not as conflicting as it appears to be.

SUMMARY

I. By means of a special method of staining, developed by the writer, the following observations on *Bacterium tumefaciens* are made. First, a very marked variation in size in young cultures, varying from 0.6 to 3.6 μ in length, and 0.3 to 1.0 μ in width. Second, the presence of many club-shaped rods in young cultures, which appear heavily stained and homogeneous by ordinary staining methods and which are found to consist of plump rods with extensions or buds from one end, by the special staining method. These buds comprise the narrow portions of the clubs and are found to possess a heavily staining interior body which is found extending at first into the main body of the rod. The process is stated to be comparable to the budding of yeasts. Third, the presence of heavily staining, bipolar bodies in ordinary rods. Evidence is presented to show that these rods are not

spores and that no spores are to be expected in this organism. Fourth, a central body found in rods possessing one polar body, the central body being in the form of a band or of two particles opposite each other, suggesting a mitotic figure. Other evidence is presented indicating a nuclear phenomenon prior to cell division.

II. Using a Berkefeld V filter in preliminary experiments, the filtrate is found to contain small rods, which when transferred give rise to characteristic growths of *B. tumefaciens*. Checked with a culture of *Bacillus amylovorus*, a small rod former but possessing no tiny buds or minute rods, the filtrate remained sterile. Attention is called to the bud-like processes described by Barnard as occurring in the virus of various tumors and comparison is made with this and the bud-like extensions on rods of *B. tumefaciens*.

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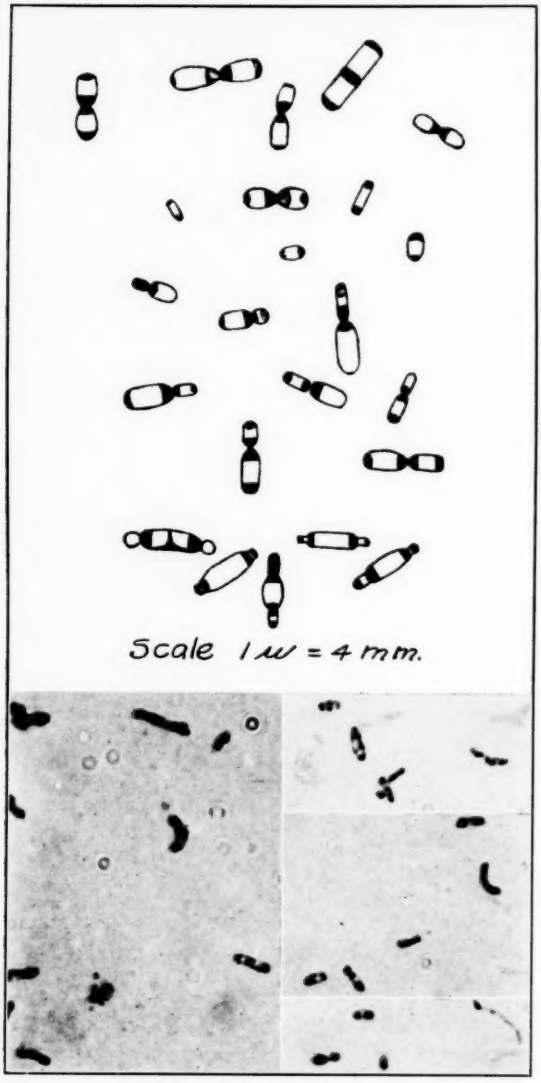
LITERATURE CITED

1. **Barnard, J. E.** The microscopical examination of filterable viruses associated with malignant new growths. The Lancet 219: 117–123, *2 pl., 6 figs.* 1925.
2. **Bergey, D. H.,** *et al.* Bergey's manual of determinative bacteriology. 1923.
3. **Burrill, T. J.** A disease of broom-corn and sorghum. Proc. Eighth Ann. Meeting Soc. Promotion Agr. Sci., p. 30–36. 1887.
4. **Elliott, Charlotte.** Halo blight of oats. Jour. Agr. Res. **19**: 139–172, *pl. c* and *26–35.* 1920.
5. ——. A bacterial stripe disease of proso millet. Jour. Agr. Res. 26: 151–159, *pl. 1–4.* 1924.
6. **Friedemann, U., & Magnus, W.** Das Vorkommen von Pflanzentumoren erzeugenden Bacterien im kranken Menschen. Ber. Deuts. Bot. Gesell. **33**: 96–107, *pl. 4.* 1915.
7. **Gye, W. E.** The aetiology of malignant new growths. The Lancet 219: 109–117, 20 charts. 1925.
8. **Levine, Michael.** Morphological changes in *Bacterium tumefaciens.* Science **62**: 424. 1925.
9. **Manns, Thos. F.** The blade blight of oats, a bacterial disease. Ohio Agr. Exp. Sta. Bull. 210: 91–167, *pl. 1–15.* 1909.
10. **Ricker, A. J.** Some relations of the crown-gall organism to its host tissue. Jour. Agr. Res. **25**: 119–132, *pl. 1–5.* 1923.
11. **Rosen, H. R.** The number and arrangement of flagella of the fire blight pathogen, *Bacillus amylovorus.* Mycologia **18**: 23–26, *pl. 3–4.* 1926.
12. **Smith, Erwin F., Brown, Nellie A., & Townsend, C. O.** Crown-gall of plants: its cause and remedy. U. S. Dept. Agr. Bur. Pl. Ind. Bull. 213 1911.



BACTERIUM TUMEFACIENS

13. ——. Bacterial Diseases of Plants. 1920.
14. ——. Some newer aspects of cancer research. Science **61**: 595–601. 1925.
15. **Waite, M. B., & Siegler, E. A.** A method for the control of crown-gall in the apple nursery. U. S. Dept. Agr. Circ. 376: 1–8, *fig. 1–7*. 1926.
16. **Walkden, H.** The isolation of the organism causing crown-gall on *Chrysanthemum frutescens* in Britain. Ann. Bot. **35**: 137–138. 1921.

EXPLANATION OF PLATES

PLATE 24 (upper)

Bacterium tumefaciens stained heavily to show the flagella. Upper two right-hand figures magnified about 1500 times, lower right-hand and upper left-hand figure about 1200 times. While somewhat heavily stained, some stubby club forms may be observed, consisting of a small bud-like process extending from one end of the rod.

PLATE 24 (lower)

B. tumefaciens showing the common bipolar bodies, also bud-like extensions from one end, and heavily staining bodies towards the centers of some of the rods. Upper left-hand figure magnified about 1500 times, the two upper right-hand figures about 1200 times, and the remaining figures about 1000 times.

PLATE 25

Upper figures drawn to scale from stained preparations representing young, vigorously growing nutrient agar cultures. Lenses used 1.5 mm. Zeiss apochromatic objective, and 10 planoscopic ocular. Counterparts of almost all the figures can be seen in the photomicrographs of the previous plates and some on this plate. For explanation of various figures, see text.

Lower figures showing bipolar bodies, buds or extensions from the ends (especially lower right-hand figure), central bodies suggesting mitotic figures (middle right-hand figure), and the extremely small size of some of the rods (note the tiny rod with the flagellum in the lower left-hand figure, and compare this in size with the bipolar rod to the right). Magnified about 2000 times.

TWO SPECIES OF PHYSALOSPORA ON CITRUS AND OTHER HOSTS

NEIL E. STEVENS

(WITH 2 TEXT FIGURES)

The following discussion of two species of *Physalospora* known to occur in the tropics forms a part of our studies of those fungi whose pycnidial stages are commonly referred to the genera *Diplodia* and *Sphaeropsis*. It is published in the hope that it may stimulate further collecting of these fungi, particularly their perfect stages, by investigators who are located in or may visit tropical countries.

A PHYSALOSPORA WITH BROWN ASCOSPORES

To place in the genus *Physalospora* a fungus with colored ascospores is of course to do violence to the established ideas of that genus. A careful study of the fungus about to be described is sufficient, however, to convince even the most sceptical that it is genetically related to *Physalospora malorum*. This fact furnishes further evidence that such distinctions as spore color in the separation of genera are in some cases highly artificial.

The fungus under consideration was collected by the writer on cut branches of cultivated *Citrus* at Herradura, Cuba, during January, 1925, and a few days earlier on cut branches of unidentified hosts, certainly not *Citrus*, at Santa Lucia Bay and Cape San Antonio, Cuba. The perithecia of this fungus are usually 225 to 250 μ in diameter, and are scattered singly in the bark of the host. When cut across, especially before the spores are fully mature, the contents have the snow white appearance which is so characteristic of the genera *Botryosphaeria* and *Physalospora* and which makes these fungi so easy to distinguish and collect. When the ascospores are mature, however, they are brown in color and the center of the perithecium then appears dark even under the hand lens (FIGURE 1, *A*) though the paraphyses and the surrounding tissue of the perithecium still remain white. Perithecia

ASCOSPORE MEASUREMENTS OF *Physalospora* SPECIES

TABLE I. LENGTHS

	Number of Spores Having a Given Length in Microns																			
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Total
P. fusca						9	15	14	16	16	14	13	2	1						100
P. rhodina (from *Persea*)		3	2	2	9	18	18	12	11	11	7	2	1	1	1	1	1			100
P. rhodina (from *Gossypium, Hicoria, Ilex, Liquidambar, Quercus,* and *Vitis.* See (1) p. 197)	3	19	13	33	32	32	91	46	138	94	70	99	54	32	15	3	9	2	2	787

TABLE II. WIDTHS

	Number of Spores Having a Given Width in Microns											
	7	8	9	10	11	12	13	14	15	16	17	Total
P. fusca					1	5	35	43	14	2		100
P. rhodina (from *Persea*)				6	14	16	31	24	6	3		100
P. rhodina (from six hosts)	1	10	31	90	115	194	159	109	67	10	1	787

TABLE III. RATIOS OF LENGTH TO WIDTH

	Number of Spores Having a Given Ratio of Length to Width							
	2	2.5	3	3.5	4	4.5	5	Total
P. fusca	29	69	2					100
P. rhodina (from *Persea*)	34	55	11					100
P. rhodina (from six hosts)	92	418	219	40	14	3	1	787

of a species of *Physalospora* having white ascospores are shown in "B" and of a species of *Botryosphaeria* (probably *B. Ribis*) in "C." Both these specimens are on avocado (*Persea*) and were collected in Cuba during January, 1925.

Asci of the brown spored *Physalospora* measure from 120 to 150 microns in length and are typically eight-spored (see FIGURE 2, *F* and *G*). The ascospores are one-celled, brown, and measure, as shown by TABLES I–III, 29–37 × 11–16 μ, mostly (more than half) 31–34 × 13–14 μ. The ascospores of this fungus are readily distinguished from those of any other known species of the genus by their brown color.

Under the conditions of our culture work the germination of these ascospores is of the same type as that described and illustrated for *P. malorum* and other species of the genus,[1] that is, a single germ tube is produced which develops to many times the length of the spore before it branches (**10**). Pycnospores were readily produced in the single ascospore cultures under the conditions described in our earlier papers (**10** and **13**). As will be apparent (FIGURE 1, *D* and *E*, and TABLES IV–VI) the pycnospores are very similar indeed to those of "*Diplodia gossypina*" and to those of the *Citrus* stem-end rot fungus which has usually been called *Diplodia natalensis*. The pycnospores of all three species when young are unicellular and hyaline with a thick wall; they later become dark colored, generally uniseptate and the wall shows characteristic irregular longitudinal grooves or markings. The measurements given in TABLES IV–VI show that pycnospores of the brown spored *Physalospora* agree closely in size and appearance with those which have been called "*D. natalensis*" and "*D. gossypina*." There is, however, some variation in the size of the pycnospores of all of these fungi, the significance of which is not yet evident but which may be associated with the host or the condition of growth. With the information now available at least it is apparently not practicable to distinguish the pycnospore stages of these fungi.

Since no fungus at all corresponding to the brown spored

[1] Most of the papers to which reference is made are cited in "The Life History and Relationships of *Diplodia gossypina*." Mycologia **17**: 191–201, 1925, by the writer, and the numbers given in this text are those in the list of literature at the end of that paper.

PYCNOSPORE MEASUREMENTS OF *Physalospora* SPECIES

TABLE IV. LENGTHS

	Number of Spores per Hundred Having a Given Length in Microns														
	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
P. fusca	4	4	22	28	14	13	12	2	1						
P. rhodina (from *Citrus*)	1	3	12	8	12	18	11	14	7	5	8	1			
P. rhodina (from *Melia*)	3	4	12	22	25	24	6	2	1		1				
P. rhodina (from *Persea*)			2	2	2	6	25	19	28	10	4	2			
P. rhodina (from *Gossypium*)	2	3	6	7	7	16	14	15	8	7	10	3	1	1	

TABLE V. WIDTHS

	Number of Spores per Hundred Having a Given Width in Microns								
	10	11	12	13	14	15	16	17	18
P. fusca		11	37	33	12	6	1		
P. rhodina (from *Citrus*)		4	24	29	13	18	11	1	
P. rhodina (from *Melia*)	6	20	32	30	10	2			
P. rhodina (from *Persea*)			4	24	30	28	14		
P. rhodina (from *Gossypium*)	1	1	10	28	28	22	5	4	1

Physalospora here described can be found in the literature, the writer is under the necessity of designating it as a new species, *Physalospora fusca*.

Physalospora fusca sp. nov.

Type specimen (No. *1003*) collected on *Citrus* at Herradura, Cuba. January 1925.

Pycnidia simple or compound; pycnidial stromata black, 1 × 2 mm. in diameter; pycnospores at first one-celled and hyaline, becoming dark brown and septate, usually with irregular longitudinal striations, 20–28 × 11–16 μ, mostly (more than half) 23–25 × 12–13 μ.

Perithecia scattered to gregarious, black externally, 225–250 μ in diameter; asci typically eight-spored, clavate, 120–150 μ long; ascospores one-celled; 29–37 × 11–16 μ, mostly (more than half) 31–34 × 13–14 μ, brown when mature.

THE PERFECT STAGE OF THE CITRUS STEM-END ROT DIPLODIA

That the perfect stage of the *Citrus* stem-end rot *Diplodia* is a *Physalospora* closely related to *P. malorum* was proven about a year ago (**13**). The ascospore material secured on *Citrus* at that time was too scanty to permit adequate description of the fungus. During a short collecting trip in Cuba in January, 1925, however, the writer obtained abundant ascospore material of this fungus on avocado (*Persea*) and *Rosa* sp. at Santiago de las Vegas. From a study of this material it appears that the perithecia of this species average about 300 to 250 μ in diameter, are scattered singly in the bark and show in section the same snow white appearance as those of *P. malorum*. (See FIGURE 1, *B*.) Asci of this species measure from 90 to 120 μ in length and are 20–25 μ in width at the widest place. They are typically eight-spored though in some specimens asci containing but four spores are by no means rare. Ascospores are one-celled, hyaline and, as shown (TABLES I–III), those from *Persea* measured 25–40 × 10–16 μ, the largest number being 30 × 13 μ. The pycnospores, as shown (TABLES IV–VI, and FIGURE 1, *D*), agree closely in size and appearance with those of *P. fusca*.

Smaller numbers of ascospores were obtained from rose but those measured were of much the same size as those on avocado,

the largest number being 31 × 11 μ in size. The fungi from these two hosts agreed in all cultural characters including good growth at 37° C. and the chromogenesis on potato dextrose agar at this temperature.

Comparison of the spore measurements given in TABLES I to VI and of the plates in this and the earlier paper show that the species of *Physalospora* common on cotton, oak, and other hosts in the southeastern United States and the one here discussed on avocado and rose are so similar in both their perithecial and pycnidial stages as not to be clearly separable by morphological characters. Hitherto the writer has been inclined to confine the name *Diplodia natalensis* to the form which grew readily at 36–37° C. and showed chromogenesis on potato dextrose agar at that temperature, because that seemed to be the most common in the cultures from rotten citrous fruit from Florida turned over to him for study by Fulton and Winston (**13**), and because Fawcett [2] in his careful studies of the temperature relations of "*Diplodia natalensis*" also had the high temperature form.

Not all the cultures of "*Diplodia*" from *Citrus* and other hosts which are able to grow at 36–37° C., however, show chromogenesis at this temperature. Moreover, the low temperature form is now known to occur on *Citrus* as well as other hosts. Among the collections made on *Citrus* by the writer at Santiago de las Vegas, Cuba, was a *Physalospora* which would not grow at temperatures above 32° C. yet agreed in all morphological characters with the high temperature form and produced pycnospores indistinguishable from those which we have hitherto called *D. natalensis* and *D. gossypina*.

Moreover, Fulton reports (unpublished material) varying percentages of the "low temperature form" in cultures from decayed citrous fruit. These are no doubt largely the fungus having hyaline ascospores and common on many hosts, though it is of course possible that *P. fusca*, which also does not grow above 32° C., may cause decay of citrous fruit in the regions where it occurs. Further study may even prove that *P. fusca* occurs in

[2] **Fawcett, H. S.**—The temperature relations of growth in certain parasitic fungi. University of California Publications in Agricultural Sciences **4**: 183–232. 1924.

South Africa and that in describing *D. natalensis* Pole Evans may have been dealing with the pycnidial stage of *P. fusca*.

In view of these facts, the wisest course at present seems to be to consider the high temperature fungus merely as a variety, and to consider as a single species the material which is common on many hosts and apparently indistinguishable on morphological grounds.

Since many of the species of *Physalospora* hitherto described in this country have little to distinguish them except the hosts on which they were collected and since we now know that certain species at least are common to many hosts, the name to be applied to the material here considered must be chosen more or less arbitrarily.

Physalospora rhodina (Berk. & Curt.) Cooke agrees well in morphological characters with the fungus which we are studying. It was collected on rose by M. A. Curtis in a part of North Carolina where our fungus is common on many hosts. This name will accordingly be applied to the fungus under consideration.

PHYSALOSPORA RHODINA (Berk. & Curt.) Cooke, Grevillea **17**: 92.

Type specimen collected by M. A. Curtis on *Rosa rubiginosa* in North Carolina. In Herb. Cooke, Kew.

Synonyms. It is not possible to give even an approximately complete synonymy at this time but the name is here used to include *Diplodia natalensis* as used by Fawcett and other American investigators, as well as *Physalospora gossypina* and *Diplodia gossypina* of our earlier papers.

Pycnidia simple or compound, varying in size and complexity with the bark of the host, from 1 to 6 or 7 mm. in diameter; pycnospores at first one-celled and hyaline, becoming dark brown and septate usually with irregular longitudinal striations; 20–33 $\mu \times 10$–18 μ, mostly (more than half) 24–28 $\mu \times 12$–15 μ.

Perithecia gregarious, black, 250–300 μ in diameter; asci typically eight-spored, 90–120 μ long, ascospores hyaline; 24–42 $\times$ 7–17 μ, mostly 30–35 $\times$ 11–14 μ.

CULTURAL CHARACTERS OF THE TWO SPECIES

On many culture media *P. fusca* and *P. rhodina* are very similar in appearance. Under the conditions of our work,

however, in which the cultures are kept in a well-ventilated greenhouse where the temperature varies from 55–60° F. to 85–90° F. and the humidity is fairly high, due to the regular watering of the green plants which occupy about two thirds of the space,

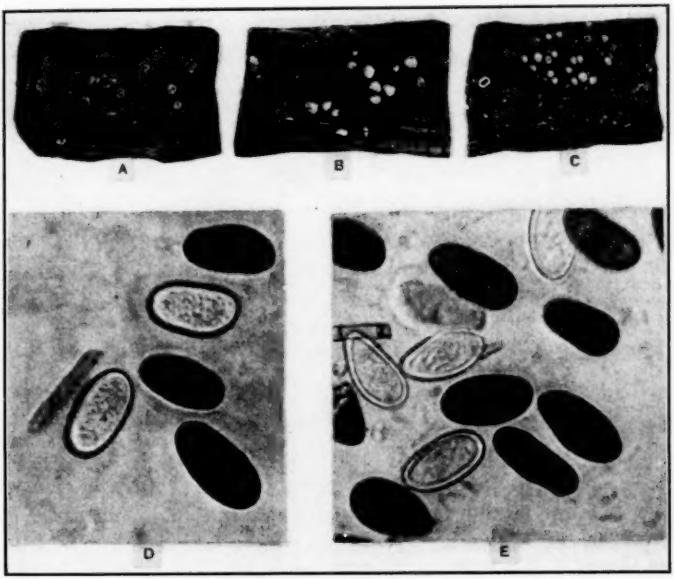


FIG. 1. A, cut perithecia of *Physalospora fusca* on *Citrus;* B, *Physalospora rhodina* on avocado (*Persea*); C, *Botryosphaeria* sp. (probably *Botryosphaeria Ribis*) on *Persea*. × 8; D, pycnospores of *Physalospora rhodina* produced in culture from single ascospores; E, pycnospores of *Physalospora fusca* produced in culture from single ascospores. × 520.

P. fusca has fruited earlier and more abundantly than *P. rhodina*. On corn meal in flasks both species produce what Earle and Rogers call "stromatic masses," that is, little mounds of mycelium in which the pycnidia are borne. These masses, however, are much smaller and more evenly distributed in *P. fusca* than in *P. rhodina* so that in mature fruiting cultures on corn-meal flasks, usually four to six weeks old, *P. fusca* much more resembles *P. malorum* than *P. rhodina*. (FIGURE 1, *D* and *E*.)

HOST RELATIONS OF THE SPECIES OF PHYSALOSPORA

No one of the species of *Physalospora* now recognized appears to be confined to a single host. We have collected *P. malorum* on over twenty host species, *P. rhodina* on nine host species and *P. fusca* on at least two. The relatively much smaller number of hosts on which the latter species have been found is very likely due to the smaller number of collections it has been possible to make. What seems to be the pycnidial stage of *P. rhodina* has, as will be pointed out below, a wide distribution both geographically and in its host relationships. So far as the writer has been able to determine from a study of the literature and from personal conversations it is the unanimous opinion of those who have studied the tropical and subtropical species of "*Diplodia*" in the field that they are not confined to particular host species but may occur on a great variety of hosts.

PHYSALOSPORA AND BOTRYOSPHAERIA ON CITRUS AND ROSA

The difficulty of identifying and satisfactorily studying the species of *Physalospora* is greatly increased by the fact that, as just mentioned, no one of the species now recognized is restricted to a single host, and in many cases two species are known to occur on the same host. For example, all three of the species of *Physalospora* which we have studied have been found on *Citrus*. *P. malorum* was collected on *Citrus* by Fulton in Alabama, *P. rhodina* by Stevens at Satsuma, Florida, and Santiago de las Vegas, Cuba, and *P. fusca* at Herradura, Cuba. At least one species of *Botryosphaeria* also occurs on *Citrus*, as the writer has received from Bowman a culture isolated from a rotten orange from California which proves to be a *Botryosphaeria* very similar to *B. Ribis*.

Somewhat similar conditions apparently obtain on many hosts. For example, we already have both *P. rhodina* and *P. malorum* on rose and some time ago found that *Botryosphaeria Ribis chromogena* not only occurred on but caused a disease of certain varieties of rose.

TABLE VI. RATIOS OF LENGTH TO WIDTH (PYCNOSPORES)

TABLE VI. RATIOS OF LENGTH TO WIDTH (PYCNOSPORES)

	Number of Spores per Hundred Having a Given Ratio of Length to Width			
	1.5	2	2.5	3
P. fusca	28	70	2	
P. rhodina (from *Citrus*)	19	81		
P. rhodina (from *Melia*)	4	92	4	
P. rhodina (from *Persea*)	30	70		
P. rhodina (from *Gossypium*)	31	59	10	

TABLE VII

LIST OF SPECIMENS OF *Diplodia* WHICH HAVE BEEN RECEIVED FROM OUTSIDE THE UNITED STATES, AND WHICH AGREE WITH *P. rhodina* IN THE CHARACTER OF THEIR PYCNOSPORES, IN THEIR ABILITY TO GROW AT 37° C. AND TO TURN POTATO DEXTROSE AGAR RED OR PINK AT THAT TEMPERATURE

Number	Host	Locality	Collector	Average Size of Pycnospores from Host	Average Size of Pycnospores in Pure Cultures
190	*Mangifera indica*	Poona, India	S. L. Ajrekar	19.6 × 11.9	22.6 × 12.8
191	*Albizzia*	" "	" " "	19.5 × 11.1	21.1 × 12.0
216	Sweet potato tuber	Pusa, India	F. J. F. Shaw	22.8 × 13.6	23.3 × 12.6
220	*Cajanus indicus*	Trinidad	F. Stell		27.8 × 14.0
227	Young tea plant	Peradeniya, Ceylon	T. Petch	20.9 × 11.4	24.3 × 12.5
228	Pod of *Hevea brasiliensis*	" "	" "	23.6 × 13.7	27.0 × 14.1
231	Diseased bark of *H. brasiliensis*	" "	" "	23.4 × 13.1	27.4 × 13.8
234	*Cinnamonium zeylanicum*	Southern Province, Ceylon	" "	25.0 × 13.9	32.7 × 12.9
260	Lime twigs	Trinidad	F. Stell		27.8 × 14.6
290	*Grevillea* seedling	Uganda. Africa	W. Small		29.6 × 13.6
305	Cocoa pod	Federated Malay States	A. Thompson		27.9 × 13.7
306	" "	" " "	" "		28.4 × 14.6
308	*Hibiscus*	" " "	" "		28.5 × 14.0
410	Strawberry fruits (*Fragaria*)	Santiago de las Vegas, Cuba	B. O. Dodge		27.0 × 13.5

THE PROBABLE DISTRIBUTION OF PHYSALOSPORA RHODINA

In view of the close similarity of the pycnidial stages of *P. rhodina* and *P. fusca*, the writer would hesitate to attempt the identification of any of these species from dry pycnospore material alone. As suggesting, however, the distribution of *P. rhodina* it may be well to record the host and locality from which we have received viable material for study (TABLE VII). Many of these specimens or cultures have been received through the courtesy of Dr. E. J. Butler, of the Imperial Bureau of Mycology.

Only those fungi are here listed which grew readily in culture at 37° C. and turned potato dextrose agar pink at this temperature, a characteristic of one form of *P. rhodina* common in Florida and Cuba. The pycnospore measurements given are simple averages of the measurements of ten apparently normal spores.

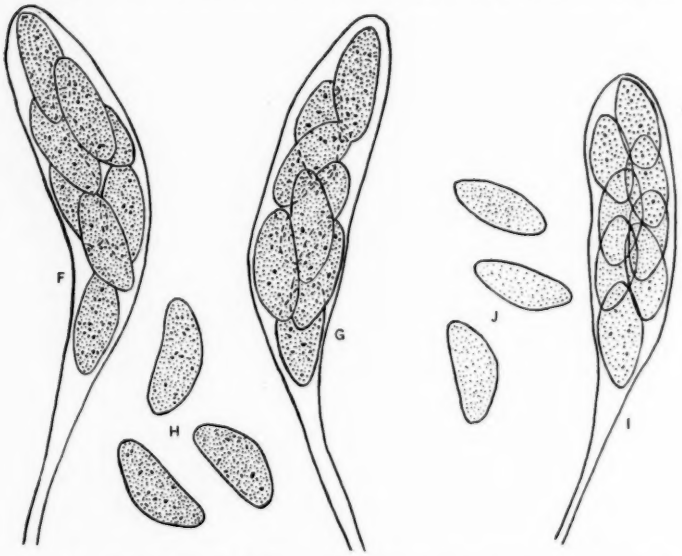


FIG. 2. F & G, asci of *Physalospora fusca;* H, ascospores of *Physalospora fusca;* I, ascus of *Physalospora rhodina;* J, ascospores of *Physalospora rhodina.* × 420.

In view of the fact that we were dealing with pycnidial material only, it did not seem worth while to measure large numbers.

What significance the variation in size of the spores from different host species or the difference between spores grown on the host and those grown in culture from the same material may have, the writer is as yet unable to say.

Whether, as seems possible, the *Diplodia* so generally parasitic on *Hevea* and *Cacao*, usually designated as *Diplodia cacaoicola*, is identical, at least in part, with *P. rhodina* can be determined only by careful comparative study. In view of the economic importance of these fungi such a study should not be long delayed.

SUMMARY

A species of *Physalospora* found on *Citrus* and other hosts in Cuba is described as *Physalospora fusca*. *P. fusca* is distinguished from other known species of the genus in having brown ascospores.

The perfect stage of the fungus which has usually been called *Diplodia natalensis* in this country is apparently identical with *Physalospora rhodina* (Berk. & Curt.) Cooke.

Physalospora gossypina as used in our earlier papers is apparently a synonym of *P. rhodina*.

What is apparently the pycnidial stage of *P. rhodina* has been received from various localities outside the United States.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

SOME FLORIDA FUNGI

AMY G. WEEDON

(WITH PLATE 26 AND 2 TEXT FIGURES)

The fungi herein described were collected by the author during a short visit to St. Petersburg, Florida, during the winter of 1923. The vicinity abounded in fungi, but time and opportunity permitted of only a small collection. Specimens of all species are deposited in the herbarium of the University of Illinois.

Hysterographium Cocos Weedon, sp. nov.

The hysterothecia appear as small black excrescences upon diseased spots of leaves, gregarious, 0.25–1 mm. long, black, carbonaceous, erumpent, superficial; opening by a long, narrow slit; asci 88–77 × 10 μ, 8-spored, spores hyaline at first, becoming dark when mature; muriform, 3–5 transverse septa with numerous longitudinal walls, 14–17 × 7–10 μ, slightly larger at one end than at the other. Paraphyses present.

On *Cocos Alphonsei*, St. Petersburg, Florida, Feb. 15, 1923, No. *4* (type).

ELLISIODOTHIS INQUINANS (Ellis & Ev.) Theiss. Ann. Myc. **12**: 73. 1914.

Asterina inquinans Ellis & Ev. Jour. Myc. **3**: 41. 1887.

Asterula inquinans Theiss. Ann. Myc. **10**: 193. 1912.

On *Sabal Palmetto*, St. Petersburg, Florida, Feb. 15, 1923, No. *3*.

This specimen agrees in all respects with the description given by Theissen, also with the specimen of Ellis and Everhart in the North American Fungi, Second Series, No. *1785*, labeled *Asterina inquinans*.

Myriangium Sabaleos Weedon, sp. nov.

The stromata appear as small, black, slightly raised excrescences, 0.1–0.5 mm. in diameter; perithecia immersed, becoming erumpent, 300–900 × 230–300 μ; asci 95 × 18–21 μ,

8-spored; spores muriform, 3–5 transverse septa and 2–3 longitudinal walls, 21–25 × 7 μ.

On *Sabal Palmetto*, St. Petersburg, Florida, Feb. 15, 1923, No. *2* (type).

The black excrescences are surrounded by a pale zone, 1–2 mm. in diameter. The spots are visible from both sides of the leaf. The asci when first released are incased in a tough sheath, forming almost spherical bodies, 24–27 × 25–28 μ. These sheaths are frequently found in the *Myriangiaceae*.

CHAETOTHYRIUM HAWAIIENSE Mendoza.

On *Citrus decumana*, St. Petersburg, Florida, Mar. 30, 1923, No. *10*.

This specimen resembles sufficiently the type specimen of *C. hawaiiense* to be considered identical with it. The fungus on the type specimen, *Morinda citrifolia*, is very scanty in amount while that on my specimen is profuse, forming a thick, sooty coating over the entire leaf. Associated with this Florida specimen are many long, stalked, beaked pycnidia. No conidia were found. Mendoza (Bull. Bishop Museum, 1925) discusses and illustrates a similar pycnidium in his description of *Antennellina hawaiiensis*.

Didymella Cocos Weedon, sp. nov.

Spots irregular in size, 3–30 × 2–5 mm. or by coalescing into long streaks which run the length of the leaf; black, epiphyllous. Perithecia scattered, carbonaceous, erumpent, 234–312 μ in diameter. Asci clavate, hyaline, 52–60 × 8–10 μ, 8-spored; paraphyses filiform, 53–70 μ long. Spores 10–14 × 3.5 μ, hyaline, guttulate, 1-septate.

On leaves of *Cocos Alphonsei* associated with *Hysterographium Cocos*, St. Petersburg, Florida, Feb. 15, 1923, No. *6* (type).

Young spots are small, and black throughout. As they get older they become ashen in the center and are surrounded by a black border 2–3 mm. wide. The tissue without the border and especially on the margin of the leaf is frequently rose colored. Spots may be visible from both sides of the leaf. Perithecia are most abundant in the ashen centers of the spots. They are for the most part on the upper surface of the leaf. The spores are slightly constricted in the middle where there is a distinct septum,

the ends are attenuated. Four guttulae are present. The lines between the guttulae give the appearance of septa, so that the spores on casual observation appear to be three-septate. It is the opinion of the writer that there is really but one septum, in which case this species would fall in the genus *Didymella*. If, however, there are three it would fall in the genus *Metasphaeria*.

Saccardo cites three species of *Metasphaeria* on *Cocos: Metasphaeria cocogena* (Cooke) Sacc. In this he says the spores are

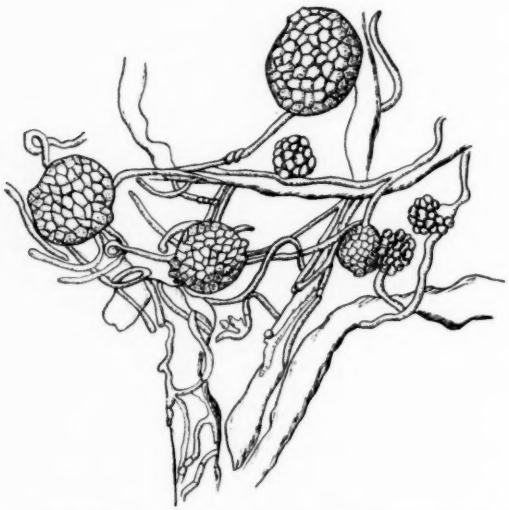


FIG. 1. *Exophoma Magnoliae*, pycnidia in various stages of development and mycelium upon trichomes of *Magnolia grandiflora*.

in two rows, tritorulosis, ends pointed, 20 × 10 μ, probably 2-septate. The specimen in question does not have spores in two rows, and they are somewhat smaller. "*Metasphaeria ? Spegazzinii* Sacc. & Trott." differs widely, the asci measuring 100 × 16–18 μ, and being aparaphysate. The third, *Metasphaeria Cocoës* Petch, does not agree either, asci being 260 × 15 μ, the spores fusoid and 85–100 × 5 μ.

UROMYCES BIDENTIS Lagh.

On *Bidens* sp., St. Petersburg, Florida, Mar. 7, 1923, No. *8*.

This specimen agrees with the description given by Sydow in the Monographia Uredinearum **2**: 3. 1910.

Exophoma Weedon, gen. nov.

Mycelium superficial, fuscous, not dematioid, not gelatinous. Pycnidia carbonaceous, free, superficial, sessile, ostiolate. Conidia hyaline, continuous.

Exophoma Magnoliae Weedon, sp. nov.

Colonies 1–3 mm. in diameter, scattered superficially on the under leaf surface or forming large, gray, mottled areas, covering the greater part of the leaf. Mycelium fuscous, branched, occasionally very slightly beaded, 3.5–5 μ thick. Young pycnidia greenish brown, becoming carbonaceous at maturity when they measure 35–53 μ in diamater; ostioles slightly papillate or cleft, 7–21 μ in diameter. Conidia hyaline, continuous, 3.5 $\times$ 1 μ.

On *Magnolia grandiflora*, St. Petersburg, Florida, Feb. 15, 1923, No. *5* (type). (TEXT FIGS. 1, 2.)

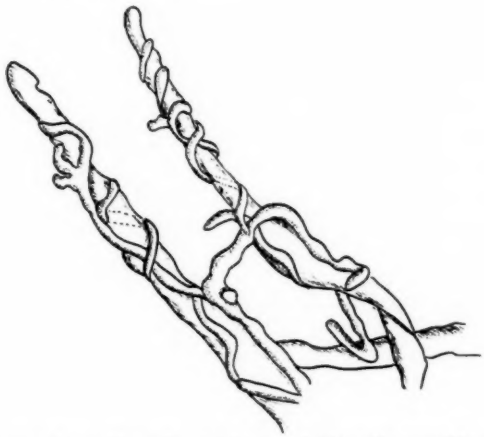


FIG. 2. *Exophoma Magnoliae*, trichomes of *Magnolia grandiflora* entwined with mycelium.

The mycelium and pycnidia suggesting relationship with the Perisporiaceae indicate that the fungus may be the imperfect

stage of one of this family. It is entirely superficial, forming a meshwork upon the trichomes, upon which it may twine loosely or heavily encrust. The vegetative mycelium is slightly lighter in color than that bearing pycnidia.

Macrophomopsis Stevens & Baechler, gen. nov.

Like *Macrophoma* but with paraphyses.

Macrophomopsis Dracaenae Stevens & Baechler, sp. nov.

Spot brown, involving a large area of the leaf. Pycnidia 155–180 μ, numerous, black, immersed, ostiolate, erumpent. Spores 22–25 $\times$ 3–14 μ, one-celled, hyaline. Paraphyses 38–55 μ in length.

On *Dracaena* sp., Guahiles, Costa Rica, July 18, 1923, No. *508*. The specimen is deposited in the herbarium of the University of Illinois.

On *Iris* sp. (cultivated), St. Petersburg, Florida, Mar. 30, 1923, No. *9*.

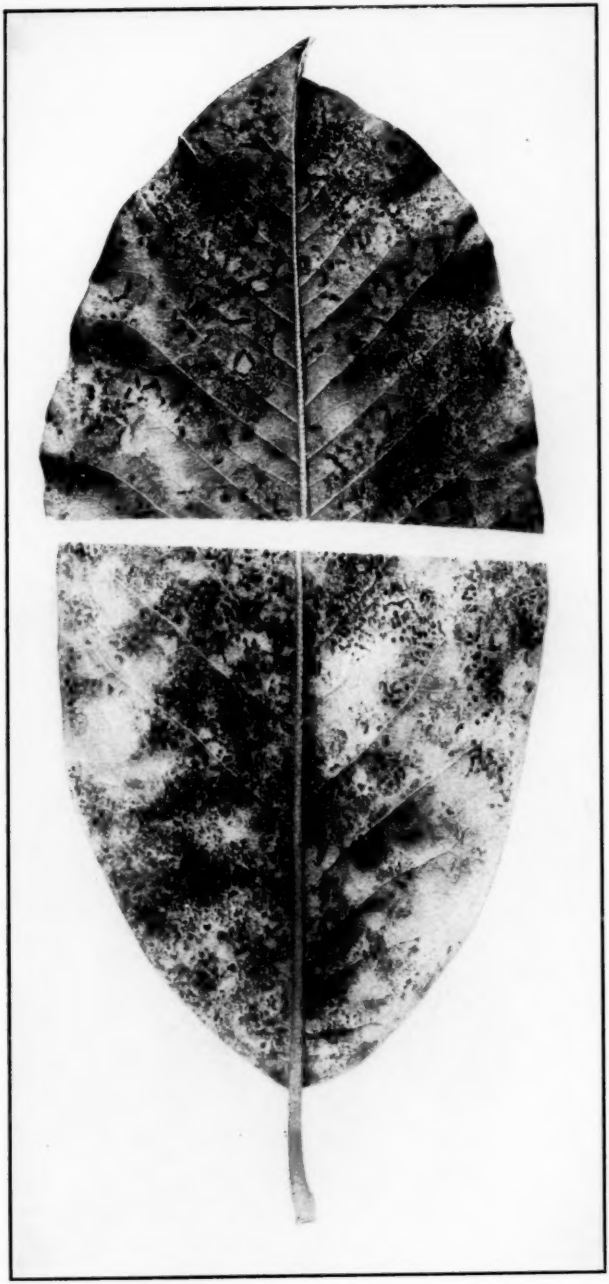
This specimen agrees identically with one found recently in Guahiles, Costa Rica and described as a new genus and species by Stevens and Baechler. Their description follows:

On crushing open a pycnidium spores of all sizes up to the maximum are found and their connection with their conidiophores is clearly evident. Between the conidiophores arise the long, slender, comparatively straight paraphyses which distinguish the genus. The characters of this fungus as well as the name suggest similarity with *Phomopsis* but the paraphyses here do not fall away as do the "stylophores" in *Phomopsis*, but remain always attached.

Heterosporium Magnoliae Weedon, sp. nov.

Colonies brownish-black, irregularly circular, 0.5–1 cm. in diameter, or consisting of large, irregular areas covering practically the entire under surface of the leaf, or scattered in bead-like, short chains, straight to circular in outline. Mycelium superficial, smooth, branched, fuscous. Conidiophores suberect, branched, echinulate, darker than mycelium, approximately 400–850 $\times$ 15–23 μ. Conidia vary greatly in size, from 14–53 $\times$ 3.5–5 μ; 1–5 or 7 septa; echinulate. (PLATE 26.)

On *Magnolia grandiflora*, St. Petersburg, Florida, Feb. 15, 1923, No. *1* (type).



HETEROSPORIUM MAGNOLIAE

This fungus differs from the typical *Heterosporium* in being superficial and perhaps for that reason should be regarded as a separate genus.

GRAPHIOLA PHOENICIS (Moug.) Poit. Ann. Sci. Nat. I. **3**: 473. 1824.

On *Phoenix dactylifera*, St. Petersburg, Florida, Mar. 8, 1923, No. 7.

This specimen agrees in all respects with the description given by Saccardo.

NORTH AMERICAN SPECIES OF SCLEROTINIA—I [1]

H. H. Whetzel

(With Plates 27–29 and 1 Text Figure)

Some ten years ago the writer agreed to undertake the preparation of a monograph on the species of *Sclerotinia* for the North American Flora published by The New York Botanical Garden. A survey of the species reported from North America up to that time led the writer to assume that the undertaking would be a relatively simple one. His limited experience in taxonomic work was also doubtless an important factor in luring him into what promises to be a long and laborious though a most interesting and enjoyable investigation.

It soon became apparent that to prepare a careful and reliable presentation of the North American species in this genus would require not only the collection and the examination in fresh condition of the morphology of as many of the forms as possible but also a comparative study of these forms in pure culture. Collecting soon uncovered undescribed species of *Sclerotinia* and species not heretofore reported from this country. The numbers of these have now increased to such an extent and the results of our field observations and culture work have presented so many new facts that a radical revision of the plans with respect to the work seems necessary.

It now appears desirable, before attempting preparation of the manuscript for the flora, to present a series of studies on groups of related species and on certain problems arising during the course of the investigations. It is proposed to publish these papers as

[1] The investigations upon which this paper is based were supported by a grant from the Heckscher Foundation for the Advancement of Research established by August Heckscher at Cornell University. The writer wishes to acknowledge also the assistance of Miss Christine Berkhout and Miss Cynthia Westcott, who as Heckscher Research Assistants have materially contributed to the success of these investigations. The photographs were taken by W. R. Fisher; the drawing was made by Miss Westcott.

rapidly as possible detailing our investigations with results and conclusions. No attempt will be made to present these studies in any logical sequence. Each paper will constitute a unit in itself and they will appear in the order in which the work on each subject is completed.

While these studies are fundamentally mycological it is perhaps our phytopathological bias which impels us to include, along with the usual morphologic and taxonomic data ordinarily comprising such monographic studies, a consideration of the life history, pathogenic effects on the suscept and cultural characters of the various species treated, in so far as our observations on these features permit. It is proposed to illustrate each species more or less fully with photographs and drawings. Previous work on the species, at least the more important papers, will be cited.

It is hoped that the presentation of these studies will stimulate greater interest among mycologists and plant pathologists in a genus of fungi all the species of which appear to be pathogenic and some of which are at least of marked economic importance. The cooperation of our colleagues both in this country and abroad is solicited. Specimens, cultures, and observations on any species of *Sclerotinia* will be especially welcome.

SPECIES ON *ERYTHRONIUM*

One of the first species to arouse our interest in the genus *Sclerotinia* was that described by Clements years ago as associated with *Erythronium albidum* in Nebraska. On a visit to the University of Nebraska in 1918 the writer examined with much interest the type material in the herbarium there and made some notes on it. The peculiar little furcate sclerotia were especially intriguing. The discovery of a form similar in many respects to *S. gracilis* Clements and associated with *Erythronium americanum* in New York has afforded the stimulus for an investigation of the forms occurring on *Erythronium*.

SCLEROTINIA GRACILIS Clements; Bessey in Webber, App. Cat. Fl. Nebr. ed. 2. 47. 1892.

This species appears to be the only *Sclerotinia* heretofore described as associated with *Erythronium*. Through the kindness

of Dr. Leva B. Walker of the Botanical Department of the University of Nebraska, we have had the opportunity to make a critical examination of Clements' type specimens and his notes and drawings. There is on the herbarium sheet in addition to the brief technical description (published by Bessey in 1892) a penciled memorandum in Clements' hand giving the reason which led him to the conclusion that his fungus had not been theretofore described. There is also a very careful pencil sketch of the apothecia and sclerotium as well as detailed drawings of asci, ascospores and paraphyses.

DESCRIPTION OF SCLEROTINIA GRACILIS CLEMENTS

It seems desirable to present here in full the description and notes just as they appear in Clements' hand on the herbarium sheet together with a photograph of the drawings (PLATE 27, FIG. 5).

"*Sclerotinia*. Cup thin, hemispherical, bright brown, margin entire, darker; stipe elongated, flexuous, thick, 1½ to 2 cm. long; asci cylindrical, attenuated at base, 150–160 × 10 μ; sporidia 8, oblong elliptical, curved, biguttulate, 26–32 × 10–11 μ; paraphyses filiform, slightly clavate at apex, somewhat branched."

"In shady woods (associated with *Erythronium albidum?*). The cups are from 2½ to 3½ mm. broad, a single stipe arising from each of the 2–3 forks of the sclerotium. Sclerotium black, thin, coriaceous, forked, ⅓ × 1½ cm."

"As Saccardo has observed no order in the arrangement of the species of *Sclerotinia*, it is impossible to say just where the specimen in hand ought to be placed."

"The species which the specimen approaches nearest are *Sclerotinia Kerneri* Wettst., *Sclerotinia baccarum* Schröt., and *Sclerotinia megalospora* Woron."

"From *Sclerotinia Kerneri* the specimen in hand is taken out by the stipe, which is at least 10 times as long, by the fact that the spores are much longer and, at the same time, more slender; by the biguttulate spores and by the sclerotium; from *S. baccarum*, it differs in the size of the cup (1⅓–½ as large), in length of stipe (only ⅕ as long), in the shape and chiefly in the size of spores, in having biguttulate spores, in the character of the asci and in the character and features of the sclerotium."

"From the peculiar sclerotium and from the large size of the spores, particularly, it seems almost certain that this must be an unrecorded species."

The label on the herbarium sheet bears the following data in Dr. Bessey's handwriting.

Sclerotinia gracilis Clements in Contrib. Bot. Dept. Univ. of Nebr. Coll. May 12, 1892. Near Lincoln. (Raymond).[2]

The type specimen on the sheet consists of two apothecia but no sclerotia. A part of this type in the Durand Discomycete Collection at Cornell University consists of a single apothecium attached to one fork of the furcate sclerotium. As far as known these represent the only specimens of this collection in American herbaria. Dr. Clements writes us (Nov. 23, 1925) that a specimen was sent to J. B. Ellis at the time the fungus was under investigation but we have not been able to locate this specimen.

Examination of a single mount from the margin of one of the apothecia of Clements' type material shows the asci, spores and paraphyses to agree in general with the above description in Clements' notes. Our measurements of 50 asci however give limits of 191–250 × 9–14.5 μ with 215.7 × 12.5 μ as the mean and 216 × 12.8 μ as the mode.[3] This it will be seen is considerably larger than the measurements given by Clements who may not have measured the full length of the ascus. Fifty spore measurements give limits of 21–29.7 × 7–9.6 μ with 23.7 × 8 μ as the mean and a mode of 21.8 × 7.8 μ, which is somewhat smaller than that recorded by Clements. Our measurements were, however, of necessity largely those of spores still in the ascus and from dried material.

NOTES.—It is highly desirable that further search be made for this fungus by collectors in the range of the host. There are in the Durand herbarium two collections (*719* and *1318*) bearing the name *Sclerotinia gracilis* Clem. both taken about Ithaca, N. Y. In the Atkinson Collection, Cornell University, are two specimens also taken near Ithaca and bearing the name *S. gracilis* Clements,

[2] Miss Walker advises me that Raymond is a little village near Lincoln in the vicinity of which the collection was made. Clements himself appears to have been the collector.

[3] Mounted in 1 per cent potassium hydroxide solution.

as determined by Durand. A duplicate of one of the Atkinson specimens, *14961*, is also to be found in the Durand Collection under his No. *2233*. All of these are reported as taken on the ground or under leaves. No mention is made of their association with *Erythronium*. None of these show furcate sclerotia and all appear to agree in every respect with *S. Erythroniae* described below.

SCLEROTINIA ERYTHRONIAE Whetzel.

On May 7, 1921, Mr. E. E. Honey and Mr. Frank Dickson, then students in our laboratory, collected a *Sclerotinia* near Tulley, N. Y. (C. U.[4] *11530*). The apothecia were taken in some abundance on a wooded hillside to the south of the swamp surrounding Labrador Lake. The cups were found protruding from the soil of little knolls in this hardwood forest. The stipes arose from rather slender fusiform sclerotia buried to a depth of one to five inches in the firm soil (PLATE 27, FIG. 2). No clue to the host plant involved was obtained.

During May of the same year and of some of the succeeding years small apothecia of similar character were collected in this and other localities near Ithaca, but from flat more or less circular or ovate sclerotia (PLATE 27, FIG. 3) lying on the surface of the soil or leaf mold under fallen leaves of the previous season (C. U. *11560* and *14761*). Cultures from these all gave on potato agar similar flat ovate or circular thin sclerotia which resembled those of certain *Botrytis* species except that they were thinner and more flattened.

On May 27, 1923, diseased leaves of *Erythronium americanum* were collected in a wood near Ellis Hollow, N. Y. Tissue plantings were made from the lesions. These gave pure cultures of a fungus forming flat black sclerotia (PLATE 29, FIG. 11). They looked much like the sclerotia of a *Botrytis* and were so labeled and filed. No conidia however ever appeared in the cultures.

On May 16, 1925, a collection (C. U. *14189*) of the apothecia

[4] Specimens deposited in the current herbarium of the Department of Plant Pathology of Cornell University will be designated with the abbreviation C. U. to distinguish them from the completed collections such as the Atkinson herbarium, the Durand Discomycete herbarium, etc., now deposited in our general herbarium.

arising from the deeply buried fusiform sclerotia was again made by Mr. Honey and the writer (PLATE 27, FIG. 1) from the same wooded hillside at Labrador Lake as those of May 7, 1921. This time careful examination of the all but completely decayed debris in which the sclerotia were imbedded together with critical observations on the surrounding spring flowers convinced us that the decaying tissues were very probably those of *Erythronium americanum*. On June 1 the writer made a second trip to this locality and all about the spot where the apothecia had been collected two weeks before, the leaves of *Erythronium* were specked with small necrotic lesions which were apparently causing the yellowing and dying of the plants. In some cases these flecks had developed into large water-soaked areas involving a large part or all of the leaf. The petioles in such cases were soft and shrivelling far into the soil. The bulbs however at this time were still firm and apparently healthy. Cultures from the diseased leaves (C. U. *14762*) brought into the laboratory gave mycelium and sclerotia typical of those obtained from ascospore sowings. Leaves allowed to rot in a moist chamber formed many flat black sclerotia which gave cultures typical of those from ascospores.

Comparison was then made of our various cultures previously obtained from *Erythronium* leaves and from unknown apothecia arising from flat sclerotia from forest soil with the result that the above reported forms when brought together proved to be so much alike as to suggest a common identity. Further comparison of cultures on standard media convinced us that all belonged to the one species, the apothecial form of which was first collected in 1921 by Honey and Dickson. The apothecial stage (C. U. *15145*) was collected in abundance in the Lloyd Preserve at McLean, N. Y. in 1926 and at Forest Home near Ithaca, N. Y. (C. U. *1564*).

The only species of *Sclerotinia* heretofore recorded as occurring in association with *Erythronium* appears to be *S. gracilis* of Clements reported from Nebraska. He found his species, however, associated with *Erythronium albidum*. Comparison of our species with his specimens and description shows it probably to be distinct.

LIFE HISTORY.—The evidence upon which the following outline of the life history of *S. Erythroniae* is based is largely circumstantial but it is believed to be substantially correct. The apothecia appear about the time the leaves of the host are full grown, in the type locality, during late April and throughout May.

The discharged ascospores fall on the leaf blades and infect them. The infections on the leaf blades are at first minute necrotic spots surrounded by a definite dark green zone (PLATE 28, FIG. 7). The leaves look speckled. The mycelium gradually spreads throughout the leaf causing a degeneration of the chlorophyl with a resulting yellowing of the leaf about the green-zoned lesions. The tissues become water soaked, then dry out becoming dirty brown and papery, finally shrivelling and falling over on the soil. The fungus spreads down the petiole, invades the bulbs and forms the typical fusiform black sclerotia. Irregularly ovate flat sclerotia, usually small in size, are formed in the rotting leaf blades on wet soil.

In the spring a single apothecium, rarely two, arises from each sclerotium. Those from sclerotia formed in the underground organs force their way up through the soil, often from a depth of five inches (PLATE 27, FIG. 2) to open and expand the cup just at the surface of the soil so that at first sight they appear to be almost sessile. Those arising from sclerotia formed in the leaves are short stalked. Infected leaves are usually abundant within a week or ten days after the apothecia appear. Ascospores discharged on the leaves of entire plants in a moist chamber and on plants in the open gave typical lesions in 4 days (PLATE 28, FIG. 7).

CULTURAL CHARACTERS.[5]—Grown on potato agar, oat agar and

[5] Standard media prepared as follows have been used in all culture work with these Sclerotinias:

POTATO AGAR.—Water (tap) 1 liter, agar shreds 18 grams, glucose 10 grams, 2 average sized potatoes, peeled, sliced and boiled in the water until tender. The glucose and agar shreds are added to the potato bouillon after it is filtered. The mixture is then autoclaved or held in a steam cooker until the agar is dissolved. The sediment is then settled out in a large funnel held in a steam cooker. After hardening the sedimented apex of the cone is cut away and the clear agar remelted and tubed or put in small flasks for pouring plates.

OAT AGAR.—Water (tap) 1 liter, Quaker Rolled Oats 35 grams, agar shreds

nutrient agar, this species exhibits the following characteristics. Only sclerotia and microconidia are produced, the latter appearing sporadically on all the media especially in old cultures on potato agar, the former only on the potato and oat agar. Small aborted sclerotia appear rarely on nutrient agar in test tube slants. On nutrient agar the fungus causes a strong discoloration of the medium giving it a dirty reddish-brown color when held against a white background and growth is relatively slow compared with that on the other two media. Sclerotia are generally larger but fewer on oat agar than on potato agar (PLATE 29, FIG. 11–12), strikingly so in petri dish cultures.

The sclerotia produced in culture are like those formed in the rotting leaves but are decidedly different in form from that which they assume in the host organs in the soil. In general they are more or less circular in outline like those produced in the infected leaf blades, averaging around 3 to 5 mm. in diameter though frequently very small ones, 1 mm. in diameter, are produced. Along the margins of the test tube slants and about the margins of the petri dish cultures elongated fused sclerotia are commonly formed next to the glass (PLATE 29, FIG. 15). There is also some tendency for the sclerotia on the surface of the slant or plate to fuse (PLATE 29, FIG. 10, 12 and 16). The distribution pattern on potato agar is indefinite, that is, the sclerotia are not arranged in any definite order but are irregularly scattered over the surface (PLATE 29, FIG. 11 and 13). On the oat agar, especially in petri dish cultures, the sclerotia are commonly more or less zonate in arrangement (PLATE 29, FIG. 10 and 12). The sclerotia in normal cultures are deep black, dull, that is, scarcely or not at all polished, thin and flat or slightly convex. They are usually superficial but are sometimes immersed in the media particularly in petri dish cultures on potato agar. No appressoria have been observed and no conidial structures other than the microconidia already referred to are produced. The microconidia are typical of *Sclerotinia*.

14 grams. The oats are steeped for half an hour in water, the agar shreds added and held in autoclave or steam cooker until melted. The sediment is then settled out as above described.

NUTRIENT AGAR.—Water (distilled) 1 liter, Leibig's beef extract 3 grams, peptone 5 grams, agar shreds 14 grams. These are heated together until the agar is melted and then filtered through filter paper or absorbent cotton.

In old strains, that is, those that have been carried in culture for several years, the sclerotia tend to form slowly as compared with recently isolated cultures. They are usually larger, less abundant and remain flesh colored for a long time, often failing to completely color, the black or brown color appearing only around the edge of the sclerotium, the center remaining white or flesh colored and often decked with a distinct white hyphal weft (PLATE 29, FIG. 14).

Sclerotinia Erythroniae Whetzel, n. sp.

Apothecia slightly elevated above the surface of the soil, 3–5 mm. broad, cupulate to broadly shallow saucer shaped, finally flat or convex with reflexed margins, fleshy (PLATE 27, FIGS. 1, 2 and PLATE 28, FIG. 6). Inside of disc at first Prout's brown (R.[6])

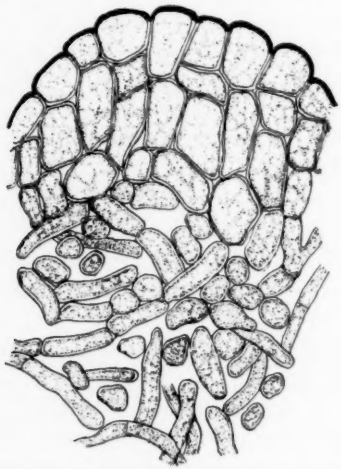
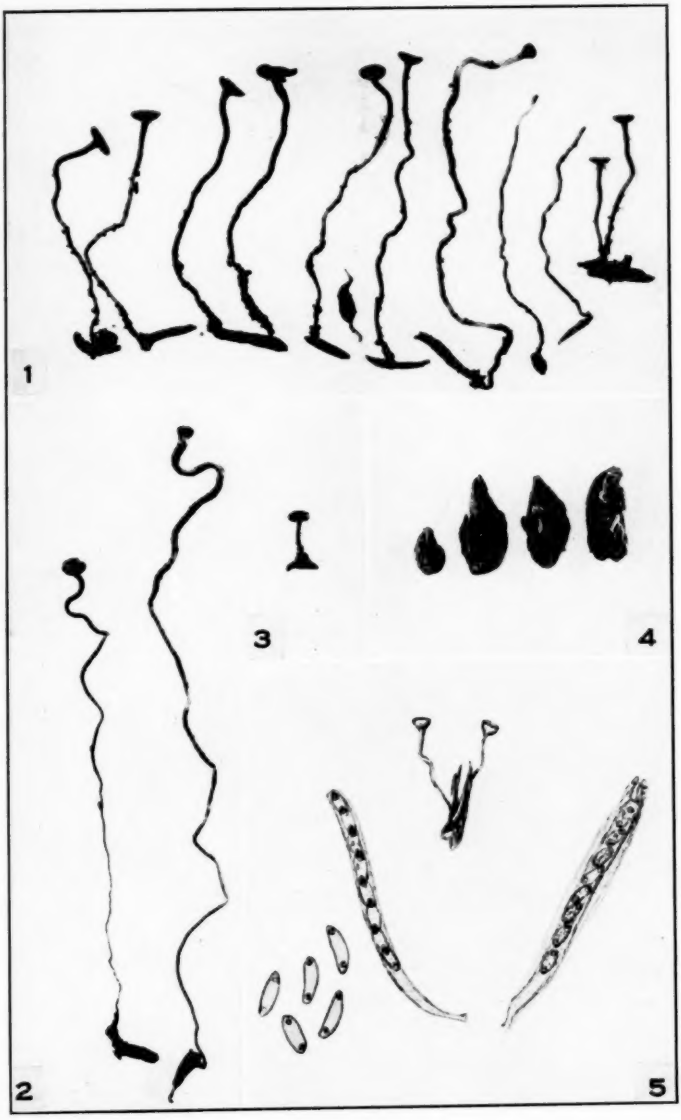


FIG. 1. Cross section of a portion of the sclerotium from the type strain (C. U. *14189*) showing structure of medulla and rind.

becoming a dark seal brown (R.[6]) with age; under surface lighter colored. Stipes often very long up to 5 or 10 cm. (PLATE 27, FIGS. 1 and 2), about 1 mm. at base of the cup and gradually attenuated toward the sclerotium, tough. Arising from deeply buried, usually more or less pointed black sclerotia, to which they are firmly attached, or from flat, ovate sclerotia in leaf mold on the surface of the soil in which case the stipes are short (PLATE 27, FIG. 3).

[6] R. = Ridgway's Color Chart.



SCLEROTINIA

Asci cylindric, attenuated toward the base. One hundred measurements give limits of 200–275 × 10–15 μ, mode 250 × 12 μ, mean 240 × 12 μ. Ascospores long elliptical, rarely inequilateral, often guttulate, one hundred measurements give limits of 20–30 × 7–10 μ, mode 25 × 8.8 μ, mean 25 × 8 μ. Paraphyses slender filiform of nearly uniform thickness throughout, or slightly swollen toward the apex.

Microconidia have been observed only in culture (PLATE 28, FIG. 9).

Sclerotia formed in the bulbs and root stalks, simple (never branched or furcate), 5–10 mm. long, in cross section broadly loaf shaped, more or less irregular in outline, 1–1½ mm. in diameter; becoming deeply furrowed lengthwise with exhaustion of stored food, as apothecia develop; flat, irregularly circular or ovate when formed in the leaves or in culture. Structure consisting of a rather loose hyphal white medulla of uniform density, surrounded by a rather distinct rind of several layers of rather large isodiametric cells forming a pseudoparenchymatous layer, the external walls of the outer cells of which are thickened and black (TEXT FIG. 1).

Occurring among colonies of the host plant on shaded knolls or wooded hillsides, April–May; parasitic on *Erythronium americanum* Ker. Type locality Labrador Lake, near Tulley, N. Y. Common in all localities about Ithaca where sought. Not known from elsewhere.

HERBARIUM MATERIAL.—Type, Cornell Dept. Plant Pathology Herb. *14189*. Other collections in the same herbarium are *11530* and *11560* (apothecia), *12484* (diseased leaves), *14761* (apothecia), *14762* (diseased leaves), *15145* and *15164* (apothecia). In the Atkinson (Cornell Univ.) Collection are *14961* and *1787* (apothecia) and in the Durand (Cornell University) Discomycete Collection *1318* and *719* (apothecia), all labeled *S. gracilis* as determined by Durand, but undoubtedly *S. Erythroniae*.

NOTES.—While our species is no doubt closely related to *S. gracilis* Clements it exhibits certain very distinct characters especially in the form of the sclerotia which in hundreds of specimens examined have never been observed to be forked as in *S. gracilis*. The apothecia are apparently larger and more fleshy. The asci and the spores are both somewhat larger. It may here be remarked that apothecial characters are much less diagnostic in the genus *Sclerotinia* than are the sclerotia. Its

parasitic habit on a different species of *Erythronium* from that of *S. gracilis* is also to be regarded as suggesting its specific identity. Comparative studies in pure culture together with cross inoculation experiments with the two species will alone determine with certainty whether they are distinct. As opportunity for us to make such studies does not appear probable in the near future it seems best on the basis of the data at hand to consider them distinct for the present.

CORNELL UNIVERSITY,
ITHACA, N. Y.

EXPLANATION OF PLATE 27

MORPHOLOGY OF *Sclerotinia Erythroniae* AND *S. gracilis*

1. Apothecia of *S. Erythroniae*, natural size, from sclerotia naturally formed in the bulbs of *E. americanum*. C. U. *14189*.

2. Apothecia of *S. Erythroniae*, natural size, showing extreme depth to which sclerotia may be buried. C. U. *11530*.

3. Apothecium of *S. Erythroniae*, natural size, arising from flat sclerotium formed in leaf on surface of soil. C. U. *14761*.

4. Sclerotia of *S. Erythroniae* formed in pure culture on steam-sterilized bulbs of *E. americanum*. Natural size. C. U. *14762*.

5. *S. gracilis*. Photograph of Clements' sketch of the type specimens in Herbarium Dept. Botany Univ. Nebraska. Natural size. Note the furcate sclerotium.

EXPLANATION OF PLATE 28

HOST RELATIONS OF *Sclerotinia Erythroniae*

6. Young apothecia with husks of rotted bulbs still attached to two of them. The two apothecia on the left are from sclerotia formed in the leaf blade. Natural size. C. U. *15145*.

7. Leaf lesions resulting from inoculation with ascospores shot from apothecia. Natural size. C. U. *15145*.

8. Sclerotia of *S. Erythroniae* forming on the recently infected young rhizomes. The petioles by way of which the fungus entered the old bulbs have rotted away. Natural size. C. U. *15145*.

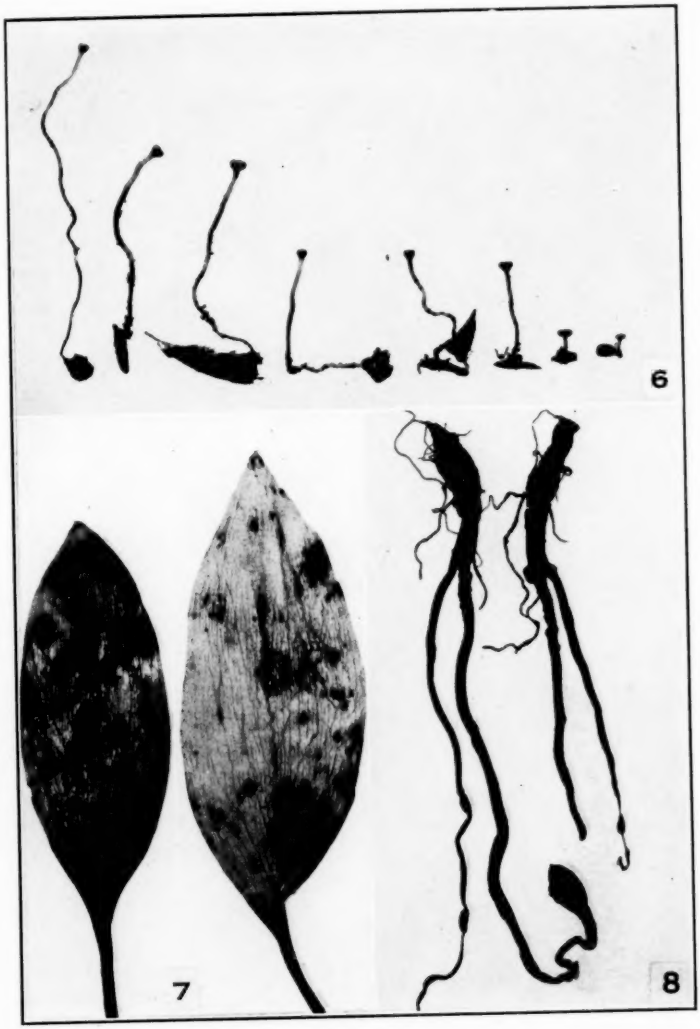
EXPLANATION OF PLATE 29

CULTURAL CHARACTERS OF *S. Erythroniae*

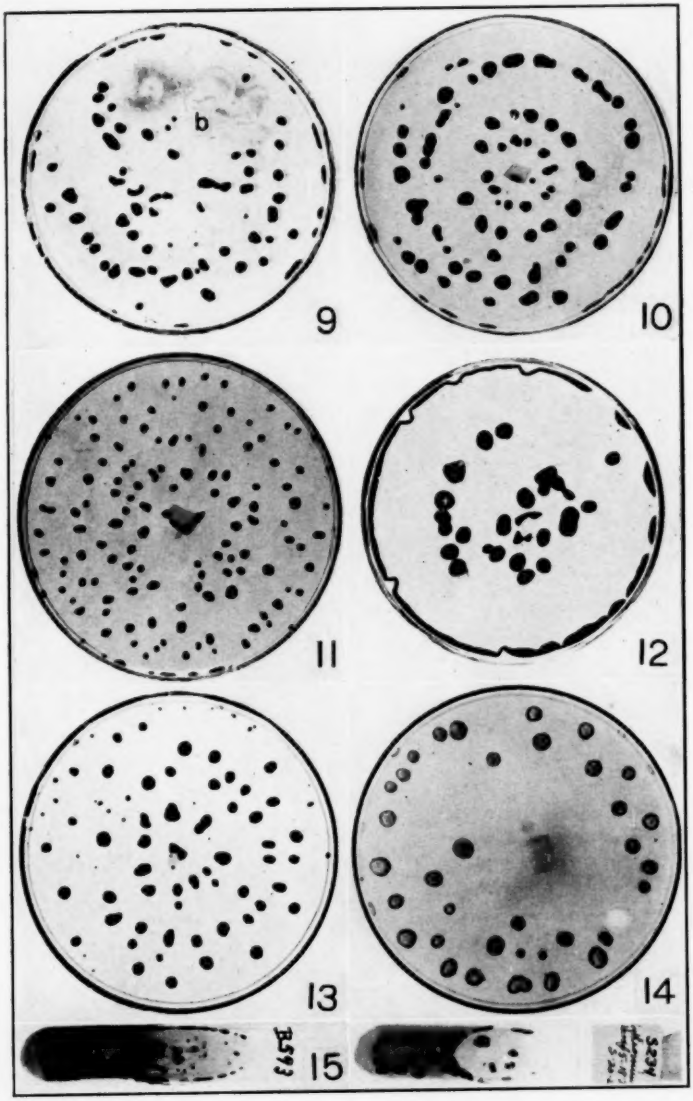
9. Original culture on potato agar from ascospores, about 3 months old. Note the abundant production of microconidia at left above b; b is a bacterial contamination; ¼ natural size. C. U. *14189*.

10. Culture of the same strain on oat agar, 3 months old. Microconidial masses appear as minute specks in the agar; ¼ natural size. C. U. *14189*.

11. Culture of strain isolated by tissue plantings from infected leaf; on potato agar, 20 days old; ¼ natural size. C. U. *12484*.



SCLEROTINIA



CULTURES OF SCLEROTINIA

12. The same strain on oat agar, 20 days old. Sclerotia just maturing; ¼ natural size. C. U. *12484.*

13. Pure culture of ascospore strain from apothecium, fig. 3; on potato agar; 3 months old; ¼ natural size. C. U. *14761.*

14. Pure culture of ascospore strain isolated in 1921; on potato agar, 20 days old. Note the hyphal weft covering the uncolored centers of the sclerotia. Sclerotia often fail to mature normally in strains kept in continuous culture for several years; ¼ natural size. C. U. *11530.*

15. Potato agar slant culture of same strain as in figs. 11 and 12, 6 weeks old; ¼ natural size. C. U. *12484.*

16. Potato agar slant culture same strain as in figs. 9 and 10, about 6 weeks old; ¼ natural size. C. U. *14189.* (Lower right-hand corner. Number omitted.)

NEW AND NOTEWORTHY FUNGI—IV [1]

JOHN DEARNESS

DISCOMYCETES

(Including Phacidiaceae and Hypodermataceae)

Pezizella minuta sp. nov.

Apothecia amphigenous, chiefly on the inner side of the needles, scattered, minute, .2–.4 mm., sessile, based in the hypoderm, at first closed then urn-shaped and finally nearly flat-dish-shaped, spreading and honey-colored when moist, incurved when dry; exciple weak, pale-brown, not quite smooth, ends of 2–3 μ thick hyphae projecting; hypothecium prosenchymatic. Asci cylindric to subclavate, nearly sessile, 8-spored, 36–60 $\times$ 8 μ; paraphyses abundant, shorter than the mature asci, 2 μ wide. Sporidia hyaline, obliquely uniseriate or partly biseriate, continuous, elliptic, 6–8 $\times$ 3½–5 μ.

On sered ends or large portions of living needles of *Pinus rigida*, Avery Creek, Pisgah, N. C., July 8, 1925. G. G. Hedgcock: *11922*.[2] *P. virginiana*, Andrews, N. C. G. G. H.: *11963*. *P. echinata*, Davidson River, N. C. G. G. H.: *24395*. *P. Taeda*, Elijay, Ga. G. G. H.: *43003*. (D: *5905*.)

The affected tissues become gray-brown and very brittle. It is a question whether the *Pezizella* does not always grow upon needles weakened or killed by other fungi particularly *Hypoderma lethale*. A *Gloeosporium*, spores 6 $\times$ 4 μ, is often present, affinity undetermined.

Pezizella ontariensis Rehm, also on pine needles, is externally similar but its sporidia are linear.

(?) DASYSCYPHA FUSCOSANGUINEA Rehm.

Parasitic on branches of *Pinus monticola* Dougl., Boundary Co., Idaho, July, 1924. J. S. Boyce: *1452*. (D: *5727*.)

[1] I, MYCOLOGIA **8**: 98–107. 1916.
II, " **9**: 345–364. 1917.
III, " **16**: 143–176. 1924.

[2] Dr. Hedgcock's serial numbers in this paper are those of the respective collections placed in the herbarium of the U. S. Bureau of Plant Industry, Washington, D. C.

"Producing cankers on trees of all ages, most severe on saplings up to 20–30 ft., sometimes killing them outright; often attacking the leader and then successive leading branches and producing a deformed or stunted tree"—J. S. B. Cankers hypertrophy the branches from 1½ to 2 times their normal thickness.

This, compared with material collected by Lagerberg on *P. silvestris* in Sweden, labeled as above and passed upon by Rehm, is paler externally, more luteous on the disk and has longer paraphyses, but on the whole is very similar. It is hardly fuscosanguineous and if it is the same species as the European one it must be much more destructive by inference from Rehm's statement of habitat "an durren aestchen"—on dry branchlets.

Marginal hairs yellow-brown, up to 160 × 4½ μ, asci to 115 × 9 μ, sporidia obliquely 1-ranked, spindle-form, 15–18 × 5–8 μ.

PHACIDIUM INFESTANS Karst. var. **Abietis** var. nov.

On leaves of *Abies concolor*, White Pine, Ore., June, 1913. J. R. Weir. Also on blighted leaves among green ones of *Abies grandis*, Lincoln Co., Mont., alt. 2900 ft., July, 1924. J. S. Boyce: *1247*. (D: *5673*.)

For comparison, I am indebted to Dr. Weir for probably authentic European material of the type on *Pinus silvestris*. The variety on *Abies* differs in not being scattered and amphigenous but strictly hypophyllous and in lineal series on each side of the midrib. The sporidia of the var., 16–23 × 6–7 μ, are smaller than the measurements, 22–35 × 8–9 μ, of Karsten's description. Exceptional sporidia in J. S. B.'s *1247* are septate. For interesting observations on the parasitism of the variety by Dr. Weir, see Phytopathology **6**: 413–4.

Phacidium abietinellum sp. nov.

Apothecia hypophyllous, the ruptured epidermis not adnate but remaining as a lid or border, .5–.75 mm., 170 μ deep; the floor 100 μ thick, the side wall of similar tissue but darker and thinner; under the hand lens not distinguishable from the preceding species. Asci 62–75 × 12–13 μ, cylindric or somewhat narrowing downwards; paraphysate. Sporidia hyaline, continuous, globose to broad-elliptic, 6–8 × 5–6½ μ.

On blighted needles among green ones of *Abies balsamea*,

Newcombe, N. Y., Aug. 22, 1924. C. R. Orton & L. O. Overholts: *9713*. (D: *5748*.)

Ph. abietinum Kunze & Schm. dehisces with four similar laciniae and, *fide* Rehm's Zur Kenntniss der Discomyceten, p. 124, has sporidia 15–17 × 4–5 μ.

PHACIDIUM BALSAMEAE Davis.

Stegopezizella balsamea Sydow.

Causing blight of leaves of *Abies lasiocarpa*, Uncompaghre Mtns., Colo., July 1911. G. G. Hedgcock: *9281*. (D: *5699*.)

Asci long-clavate, 90–120 × 10–12 μ (the type "80–100 × 10 μ"), and sporidia naviculate instead of "ovoid"; the other features are in full agreement. Sydow's genus—*Stegopezizella*—and type species are based on J. S. Boyce's *966* on *Abies concolor*, Wasco, Oregon.

PHACIDIUM PLANUM Davis.

Causing needle-blight of *Pinus flexilis* James, Palmer Lake, Colo., July 19, 1917. Hedgcock and Bethel: *26127*. (D: *5619*.)

Sporidia mostly aggregated towards the top of the asci, many of them subacute at both ends, always at the lower end, 24–34 × 3–4½ μ; in type "30–40 × 3 μ." Seems to be in *Stegopezizella* Sydow; when confluent it looks like *Rhabdocline* Sydow.

Phacidium convexum sp. nov.

Apothecia dark-brown, mostly on the inner side of the needles, developing in the mesophyll, penetrating to the bundle-sheath, erumpent through a cleft in the epidermis, less frequently raising and breaking off a flake or lid of the epidermis, issuing by a neck 80–175 μ in thickness and then expanding into a dark, convex, circular or irregularly elongate disk, 300–600 μ wide, total depth 300–350 μ, thecial layer 80–110 μ. Asci cylindric-clavate, 80–102 × 8–9½ μ; paraphyses longer, linear, sparingly branched. Sporidia hyaline, nearly horizontal or irregularly biseriate in the upper part of the asci, then uniseriate, elliptic, continuous, 7–10 × 5–6 μ.

On living needles of *Pinus rigida*, Pisgah Nat. For., N. C., June 7, 1925. G. G. Hedgcock: *24388*. Davidson River, N. C. G. G. H.: *43027*. (D: *5828*.)

Although this species has a strong hypothecium it lacks an adnate lateral exciple and is therefore hardly a true *Phacidium*. It

is in this respect similar to the two preceding species described by Dr. J. J. Davis (Trans. Wisc. Acad. Sci. **20**: 424. 1921.) On the same page Dr. Davis described another *Phacidium* in this group —*Ph. expansum* on *Picea mariana*. It is erumpent then expanding, with asci 60–80 × 7–9 μ and sporidia 9–12 × 3–5 μ.

Phacidium taxicolum Dearn. & House, sp. nov.

Apothecia black, hypophyllous, in a line on each side of the midrib, rather sharply pustulate, rising 110–170 μ above the cuticle, dehiscing by 3–4 often unequal laciniae, subepidermal, 250–340 μ at base; lateral wall and floor black, 33–40 μ thick, layer of asci and hyaline hypothecium 44–60 μ deep. Asci subcylindric, narrowing slightly downwards, 33–42 × 4–9 μ; paraphyses linear, hardly as long as the asci, not abundant. Sporidia hyaline, uniseriate, sometimes biseriate in the upper part of the ascus, navicular, mostly minutely nucleate at each end, 7–8 × 2½–2.75 μ.

On blighted leaves of *Taxus canadensis*, Blue Mountain Lake, Hamilton Co., N. Y., Sept. 1920. H. D. House. (D: *4811.*) *Phacidium Taxi* Fries is said to have small, level, grayish-black apothecia; contents undescribed.—Rehm in Rabenhorst's Discomyceten, p. 72. Dr. J. J. Davis (Trans. Wisc. Acad. **21**: 280. 1924) reported collecting this species having asci about 50 × 6 μ, mature spores lacking.

Bifusella Abietis sp. nov.[3]

Apothecia on yellowed leaves, black, nervisequious, interrupted or occupying the whole length of the midnerve, sulcate. Asci cylindric or subclavate, large, saccate, almost aparaphysate, 150–180 × 27–32 μ, 8-spored, before staining appearing to be 16-spored. Sporidia consisting of two fusoid or naviculate portions joined by a slender thread, upper 12–15 × 5–7 μ, lower 10–13 × 3–4½ μ, the joining part 12–25 × 1 μ, all in a jelly sheath reaching a width of 15 μ.

Parasitic and fruiting on year-old and older leaves of *Abies lasiocarpa*, Challis Nat. For., Bonanza, Ida., Aug. 7, 1911. G. G. Hedgcock: *9375*. Also Uncompaghre Mtn., Colo.: *9286*, *9312* and other collections. Sopris Nat. For., Colo. F. V. Keefe: *20136*. Manti Nat. For., C. F. Korstian. J. R. Weir: *6955*.

[3] This and the following six forms may be interlined in the table of Hypodermata, MYCOLOGIA **16**: 147.

On *Abies arizonica*, Gila Nat. For., N. M. Hedgcock and Long: *9900*. (D: *5700*.)

Found in exactly similar leptostromes on *Abies lasiocarpa*, at Rico, Colo., Payson and Hedgcock: *26035*, a conidial form with hyaline, 3-septate spores, 18–20 × 4–5½ μ, on long conidiophores—**Cystothyrium Abietis** n.s. it may be called—but not under conditions to warrant conclusion that it is related to the *Bifusella*. (D: *5701*.)

Hypoderma Hedgcockii sp. nov.

Apothecia amphigenous but mostly on the inner side of the needles, hypodermal, conspicuous, black, not nervisequious, somewhat convex, wide for their length, .6–1.75 mm. long by .5–.75 mm. wide; slit narrow, lips hardly ridged. Asci bag-like or sub-cylindric, narrowing downward, round at the distal end, contracted at base, sessile, 100–155 × 22–28 μ, 4-spored, seldom 3- or 2-spored, occasionally containing additional, one or more small, jelly-sheathed spores, 6–9 × 3 μ. Stratum of paraphyses mostly about one and a half times the depth of that of the asci; paraphyses jelly-sheathed, abundant, linear, summit sometimes enlarged to 3 μ in width. Sporidia hyaline, granular and guttate, oblong-elliptic, or cylindrical with rounded ends, seldom somewhat slipper-shaped, finally some of them becoming uniseptate, 20–39 × 10–14 μ. When freed from the ascus the jelly-sheath in water swells up to 42 × 30 μ.

Parasitic on needles of *Pinus caribaea*, Silver Springs, Fla., May 15, 1919. G. G. Hedgcock: *32386*. Also on other *Pinus* spp.—*P. virginiana*, Jamestown, Tenn.: *41573*, *P. clausa*, Bolton, Fla.: *25244*, *P. palustris*, Croom, Fla.: *25171*, *P. rigida*, Pisgah Nat. For., N. C.: *11904*, *P. echinata*, Murphy, N. C.: *11988*, and several other collections by Dr. Hedgcock. On *Pinus inops*, Garret Park, Md., May 26, 1891. B. T. Galloway: *1342*. (D: *5827*.)

A form on *P. echinata*, Luray, Va. (G. G. H.: *22342*), has longer and more spindle-shaped sporidia, reaching 42 μ.

This fine species is one of the most distinct of the "tar-fungi." Its *Leptostroma*, described on a succeeding page, is equally conspicuous and more common.

Hypoderma lethale sp. nov.

The affected needles are usually sered for half or more than half their length, the sered or grayish portion usually terminating basally in a brown portion, .5–2 mm. long. Apothecia hypodermal, carbonous outwardly but lacking an internal carbonous floor, amphigenous, subseriate, dull black or somewhat shining when convex, ½–1 mm., sometimes longer, up to 3 to 5 mm., narrow, .2–.35 mm., mostly not wider than the space between two rows of stomata, quite flat, sometimes depressed, convex when well filled with asci, the cleft hardly visible under the microscope. Asci 8-spored, cylindric, or somewhat narrowed below, nearly round at top, 105–120 × 20–30 μ, extreme range of length found in a single apothecium 90–147 μ. Paraphyses filiform, jelly-sheathed, longer than the asci, up to 160 μ, not curved at tip. Sporidia hyaline, continuous, rarely appearing septate or pseudo-septate, obliquely 1-seriate or partly biseriate, granular, mostly obtuse at both ends, 14–29 × 3–5 μ, including jelly-sheath 16–31 × 9–12 μ.

Severely parasitic on needles of *Pinus rigida*, Highfield, Md., July 24, 1914. G. G. Hedgcock: *15626*. At scattered stations from New Hampshire to Virginia. G. G. H.: *2832*, *8063*, *8177*, *15677*, *17790*, *24340*, *36269*, and at Long Island by R. Latham. On *Pinus virginiana*, Maryland to S. Carolina. G. G. H.: *8003*, *8414*, *11777*. On *P. echinata*. G. G. H.: *11948*, *11792*. On *P. serotina*. G. G. H.: *32360*. On *P. Taeda*. G. G. H.: *43052*. (D: *5862*.)

A *Gloeosporium* stage of this species, spores 1½–2 × ¾ μ, on short conidiophores, runs in lines along the stomata producing no macroscopic effect to mark their position. This stage may be the cause of the severe blighting of the needles. "Gray blight" is the name given to it by the Forest Service officers.

HYPODERMELLA LARICIS Tub. var. **octospora** var. nov.

Asci 8-spored, clavate, 42–75 × 17–24 μ, paraphyses as long as the asci.

On *Larix occidentalis* Nutt., St. Maries, Idaho, Sept. 18, 1911. G. G. Hedgcock: *11117*. Also *9473*, *9541* and other collections. (D: *4106*.)

The description and figures of *H. Laricis* Tub. in Tubeuf's Studien die Schuttekrankheit der Kiefer, 1901, pp. 18–21, give the asci 4-spored, 90–110 μ long, cylindric and short-paraphysate.

The material referred to in MYCOLOGIA **16**: 151 was immature compared with some of these collections.

HYPODERMELLA MONTIVAGA (Petr.) Dearn. f. **concolor.**

The form described by Petrak has black apothecia from 1–4 mm. long. In fifteen collections of affected branchlets of *Pinus contorta* made by Hedgcock and others in various parts of Wyoming and Colorado, nine of them were found to have uncolored apothecia. Some of them have the typical black apothecia only; others have both the black and uncolored; while collections numbered *22532, 22544, 22593, 26410, 26420, 26426, 26870* and Boyce *1238* in Montana have uncolored apothecia only. The uncolored sporocarps are usually shorter than the dark ones and are subject to naked-eye detection by sunken depressions, often slight circular pits, in the surface of the needles.

This form may be referred to as f. *concolor.* (D: *4921.*)

Lophodermium australe sp. nov.

Apothecia mostly on the outer side of the needles, less conspicuous, duller, narrower, longer, and flatter than those of *L. pinastri*, black, acute at the ends, so narrow as to be confined between two rows of stomata, the basal pseudoparenchyma centering along a strand of thick-walled hypoderm (sclerenchyma) and depressing the mesophyll to the bundle-sheath, usually .5–2 mm. long by .2–.3 mm. wide but exceptionally 4 or 5 mm. and in one or two examples even 10 mm. long, 125–150 μ deep, carbonized roof 50–80 μ thick, basal layer .5 mm. wide. Asci cylindric, drawn in above to an obtuse summit, of different sizes in the same apothecium and varying from 65–70 $\times$ 8–10 μ in some apothecia to 120–150 $\times$ 10–12 μ in others; paraphyses linear, abundant, sometimes much exceeding the asci and slightly enlarged at the tip. Sporidia linear, nearly as long as the asci, slightly larger at the top, 1¼–1½ μ thick, with jelly-sheath 6 μ thick.

Mature fruit on languishing or partly green needles of *Pinus palustris*, Silver Springs, Fla., Feb. 27, 1919. G. G. Hedgcock: *32146*. On *P. Taeda*, Brooksville, Fla. G. G. H.: *17436*. Also by the same collector on *P. glabra, P. echinata, P. resinosa*, mature and immature, in all over forty collections in several of the southern states. (D: *5829.*)

A *Leptostroma* with conidia 5–9 × 1 μ was frequently found in these collections.

Lophodermium Laricis sp. nov.

Apothecia on the outer side of the needles, dark-brown, sometimes surrounded by an innate dark line, short, .6–1½ × .5 mm., elliptic, not strongly testudinate, cleft narrow, lips acutely ridged; the needles are marked by dark, transverse lines. Asci 120–155 × 9–12 μ, nearly cylindric, contracted towards apex, sparsely paraphysate; paraphyses linear, some of them turned at the tip. Sporidia linear, nearly as long as the asci. Conidial leptostromes circular; conidia bacillar, 6–8 × 1–1½ μ.

On needles of *Larix Lyallii* Parl., Bitter Root Mtn., Idaho, Sept. 13, 1911. G. G. Hedgcock: *20240*. (D: *5693*.)

Externally and in dissection this is nearer *L. pinastri* than *L. laricinum*, indeed it may be a host variety of the former.

TYMPANIS BUCHSII (Henn.) Rehm.

On *Pinus monticola*, Stocking Meadows, Ida., Aug. 1923. C. R. Stillinger: *1340*. (D: *4275*.) "Causes cankers on living branches."—C. R. S.

In the probably immature asci eight large, hyaline, elliptic sporidia, 15–17 × 10 μ. These seem to break up into innumerable bacterium-like spores, 2½–3 × .75 μ. In Zur Kenntniss der Discomyceten Deutschlands, 1912, p. 205, Rehm speaks of finding in this species "unentwickelten Schlauchen" with 8 elliptic, hyaline, 1-celled sporidia, 18 × 10 μ.

PYRENOMYCETES

Dimerosporium Abietis sp. nov.

Perithecia on branching brown mycelium, densely clustered on the lower side of the leaves, dark-brown, mostly about 150 μ; hyphal appendages numerous, brown, obtuse, sometimes septate, 30–80 × 4–5 μ. Asci saccate, paraphysate, 40–60 × 21–28 μ, several in the perithecium. Sporidia conglobate in asci, hyaline, 1-septate, 27–30 × 9–10 μ.

Parasitic on the older needles of *Abies amabilis*, Red Mountain, Skamania, Wash., elev. 3300 ft., Aug. 14, 1924. J. S. Boyce: *1261*. Also on *Abies grandis*. J. S. B.: *1263*. J. S. B.: *584* (Mycologia **16**: 154) is probably this species. (D: *5653*.)

Meliola pinicola sp. nov.

Dark-brown mycelial patches on the side of the needles, sometimes surrounding them, 1–4 mm. in diameter; hyphae 8–9 μ thick, branching mostly at a half right angle; hyphopodia 25–30 μ long, head 12 μ thick. Perithecia dark brown, tuberculately-rugose, appendaged with septate, brown hyphae 8 μ thick, subglobose, flattened on the top, 180–275 μ. Asci bisporous, mostly about 50 × 30 μ. Sporidia brown, 3-septate, large, 45–60 × 10–16 μ.

On living needles of *Pinus echinata*, Pisgah Nat. For., Pisgah, N. C., July 6, 1925. G. G. Hedgcock: *24394*. (D: *5878*.)

Dimerium Juniperi sp. nov.

Subicula small, .5–.75 mm., dark, disc-like, seated mostly on the middle of the back of the leaf-scale, consisting of anastomosing, septate hyphae, 6–10 μ. Perithecia congregated on the subicula, concolorous, 80–100 μ. Asci of various shapes, saccate, mostly widest in the lower portion, 31–46 × 9–20 μ, 8-spored, aparaphysate. Sporidia often 1-seriate above and more or less crowded in the lower half, dark-brown, 1-septate, constricted, upper cell usually larger, 11–15 × 4½–6 μ, mostly 12½ × 6 μ.

On green branchlets of *Juniperus occidentalis* Hook., Big Bear Lake, Calif., Aug. 2, 1920. Ellsworth Bethel. (D: *5566*.)

EUTYPELLA SEPULTA (Berk. & Curt.) Dearn. New description.

Stromata scattered, marked externally by a small, black pustule through which short ostiola project; sometimes a black, internal line circumscribes a paler area in which the perithecia are located. Perithecia 1–4, deeply immersed in the unaltered or hardly altered wood, subglobose, separate below, united in the disk by their ostiola, 200–350 μ; ostiola sulcate in well developed examples. Asci minute, 12–16 × 3½–4½ μ, clavate, stipitate; paraphyses not numerous, several times as long as the asci. Sporidia hyaline, allantoid, nucleate at each end, 3–4 × .75 μ.

On dead stems of *Smilax* (*?*)*rotundifolia*, Orient, N. Y., Nov. 1919. R. Latham: *4031*. (D: *4439*.)

There can hardly be any doubt that this is the species on *Smilax* mentioned in the list of imperfectly known *Eutypae* in Ellis & Ev. N. Am. Pyrenom., page 507.

Guignardia Boltoniae Dearn. & Barth. sp. nov.

Perithecia scattered, covered by the cuticle, the short ostiola barely erumpent, 80–110 μ in diameter, depressed-globose, membranous, wall of a single layer of polygonal brown cells, 10–15 μ. Asci 60–75 × 16–19 μ, cylindric-clavate, short-stipitate, aparaphysate; wall 3–4 μ thick at the top. Sporidia hyaline, crowded in the asci, fusoid, subacute at the ends, 15–18 × 4½–5 μ.

On dead stems of *Boltonia diffusa*, Devol, Okla., May 8, 1923. E. Bartholomew: *8051*. (D: *5762*.)

Intimately associated with a microconidial form—**Phoma Boltoniae** sp. nov.—in similar pycnidia, spores 4–7 × 2½–3 μ; and macroconidia—**Macrophoma Boltoniae** sp. nov.—also in similar pycnidia, spores 17–22 × 5–7 μ, fusoid, subacute at the ends.

Guignardia Lonicerae Dearn. & Barth. sp. nov.

Spots reddish-gray with wide red-brown border above, pale-gray beneath, circular, 2–5 mm. in diameter. Perithecia epiphyllous, black, numerous, prominently raising the cuticle, globose at first, becoming plane or even depressed and papillate, deeply seated, 120–160 μ in diameter and nearly as high as wide. Asci aparaphysate, clavate, 48–62 × 12 μ. Sporidia hyaline, continuous, biseriate in the upper part of the ascus, somewhat pip-shaped, widest above the middle, narrowing downward, 12–15 × 6–6½ μ.

On living leaves of *Lonicera hispidula*, Alto Palo, Calif., March 16, 1918. Dr. E. T. Bartholomew. (D: *5842*.)

Mycosphaerella stromatoidea sp. nov.

Spots numerous, scattered, small, 1–1½ mm. circular, border dark, raised, surrounded by a diffuse red band about 1 mm. wide. Perithecia innate, 15–20 so crowded together at the middle of the spot as to seem stromatic, subglobose, about 100 μ wide, opening on the upper side of the leaf. Asci aparaphysate, joined below forming a ball filling the perithecium and adapting their shape to their position, mostly about 45 × 12 μ, sporidia biseriate. Sporidia hyaline, becoming dilutely colored, uniseptate, suboblong, upper cell wider and larger, 12–15 × 5 μ, not constricted.

Parasitic on leaves of *Rumex occidentalis*, Clallam Co., Wash., Aug. 8, 1924. J. S. Boyce: *1288*. (D: *5668*.)

This can hardly be fitted into *S. Rumicis* (Desm.) Cooke.

Mycosphaerella Pachystimae sp. nov.

Spots pale-gray or whitish above the leaf, dark-gray to brown below, with narrow, black, raised border, subcircular, 2–4 mm. Perithecia epiphyllous, seldom amphigenous, evenly scattered, black, innate, rupturing the cuticle stellately, when mature 125–200 μ wide, 85–100 μ deep, nearly hemispheric. Asci 75–78 $\times$ 8–9 μ, rounded above, subcylindrical, aparaphysate. Sporidia usually more or less biseriate in the upper half of the ascus, hyaline, uniseptate, somewhat constricted, often with two oil-drops in each cell, 20 $\times$ 4–5 μ.

On languishing leaves of *Pachystima Myrsinites*, Coolin, Idaho, alt. 3000 ft., July 27, 1924. J. S. Boyce: *1287*. (D: *5667*.)

Venturia lanea sp. nov.

Perithecia membranous-carbonous, in groups or loose clusters on small, fleecy, whitish patches of mycelium, 1½–3½ mm. in diameter, turbinate, centrally depressed, 180–225 μ across, 100–150 μ high, black, shining; dark-brown, spine-like bristles, 30–45 $\times$ 5–6 μ, scattered over the whole surface. Asci 35–50 $\times$ 6–8 μ, fusoid; paraphyses lacking or obscure in mature perithecia. Sporidia hyaline, medianly 2-ranked, 1-septate, 4-guttate, navicular, 8–11 $\times$ 3 μ.

On bark of *Chamaecyparis nootkatensis*, Upper White River, Wash., Sept. 1924. J. M. Grant: *6018*. (D: *5730*.)

On the bark of the same host—Alaska cypress—same date and same locality, exactly similar bristly perithecia, not on fleecy subicula but in dense clusters around minute, erumpent, dark, wine-colored auriculae suggesting the genus *Gibbera* in the sense of Ellis & Ev. N. Am. Pyrenom., page 248. The sporidia are mostly short-elliptic, enucleate, 1-septate, 6–8 $\times$ 3½–4 μ.

Diaporthe Ostryae sp. nov.

Stromata in the outer cortex, no circumscribing line, no visible effect on the cambium side of the cortex. Perithecia single or a few circinating in the bark with connivent, minute, perforate ostiola appearing as black dots through a small, .3 mm., circular rupture of the epidermis, 175–200 μ. Asci cylindric, 58–75 $\times$ 7–10 μ; paraphyses obscure. Sporidia mostly obliquely uniseriate, not appendiculate, hyaline, uniseptate, somewhat constricted, upper cell usually larger than the lower one, 15–20 μ long, commonly 18 $\times$ 4½–6 μ, upper cell usually 5½–6½ μ wide, lower end often subacute, contents homogeneous.

On dead branches of *Ostrya virginiana*, London, Ont., July 1904. Dearness: *2161*.

This is the same species as Ellis & Ev. N. Am. Fungi *3430*, distributed in 1896 under the label "*Diaporthe ostryigena*," description not published. Barth. Fungi Columb. *2019* in 1905 and Rehm's Ascom. *1989* in 1912 distributed as *Diaporthe ostryigena* Ellis & Dearn., description in Sacc. Syll. Fung. **22**: 377 and on label of Fungi Columb. *2019*, are correctly named. They are very distinct from *D. Ostryae* (N. Am. Fungi *3430*). The latter is near *D. albocarnis* Ellis & Ev. on *Cornus*.

Diaporthe (*chorostate*) **melanocarpa** sp. nov.

Stromata sparsely scattered, for the most part under lenticels, pustular, barely exposed through clefts of the epidermis, 1.75–2.25 mm. in diameter; ostiola small, cylindric, black, shining, dome-like on top but sometimes minutely stellately cleft, crowded, as many as 20 to 25 barely protruding through a cortical disc .8 mm. in diameter. Perithecia numerous, 10–26, 250 μ in diameter, coriaceous, circinating in the inner cortex, slightly imprinting the wood, the group occupying a circle 1–2.25 mm. in diameter, reaching the surface by long, slanting or nearly horizontal ostiola. Asci fusoid, 60 × 7½–9 μ; ? paraphysate. Sporidia straight, hyaline, subseriate, naviculate-fusoid, uniseptate, not constricted, 4- occasionally 6-guttate, 17–22 × 3–4 μ.

On dead branches of *Pyrus melanocarpa*, London, Ont., May 1913. Dearness: *3488*. Newcomb, N. Y., H. D. House.

Comparison with the type of *D. impulsa* (Cooke & Peck) Sacc., to which it seems nearest, shows differences in the size of the sporidia and arrangement of the asci which are not fasciculate as in *D. impulsa*. The ostiola are different in shape and not thrust thru the surface in the manner which probably suggested the specific name of the latter species.

Amphisphaeria pelorospora sp. nov.

Perithecia nearly superficial, carbonous, black, subrugulose, hemispheric to subglobose, 1–1.25 mm. in diameter; ostiola wide, 250 μ, short-papillate. Asci 300–350 × 30–35 μ, 4-spored, short-stipitate, paraphysate. Sporidia brown, 1-septate, constricted, seldom 2 additional septa without constrictions, biconic or oblong-elliptic, ends obtuse-pointed, 75–105 × 30–33 μ.

On outer rough bark of *Nyssa* trunk, Greenport, N. Y., Feb. 1924. R. Latham: *1703*. (D: *5453*.)

This fine thing seems to be the same as my copy of Ellis & Ev. N. Am. Fungi *3012* on *Nyssa* bark with sporidia reaching 102 × 33 μ, labeled *Amphisphaeria papilla* Schw. Syn. Car. No. 159. In the Ellis & Ev. description of Syn. Car. No. 159, in N. Am. Pyrenom. pp. 178–9, sporidia are hyaline and measure 30–50 × 15–18 μ. The latter can hardly be the species under notice although there is no term in Fries' description of *Sphaeria papilla*, Syst. Myc. **2**: 462, that rejects it.

Zignoella ostiolata Dearn. & Barth. sp. nov.

Perithecia scattered or concealed among the fibers, black, carbonous, rough, hemispheric, ¼–½ mm.; ostiola large and variable—papillate, urceolate or even lophiotremous. Asci clavate, paraphysate, 105–120 × 13–16 μ. Sporidia hyaline, 1- to 5-septate, variable in shape,—some acute, others rounded at the ends, constricted, 26–30 × 6–8 μ.

On dead, weathered, woody stems of *Artemisia tridentata*, Thermopolis, Wyo., June 28, 1924. E. Bartholomew: *8734*. (D: *5703*.)

Hypospila californica Dearn. & Barth. sp. nov.

Spots brown, large, 1–3 cm., irregular, unbordered, superficially similar on both sides but under the microscope the upper side of the leaf is depression-dotted over the perithecia while on the lower side their position is marked by the extrusion of the ostiola. Perithecia innate, curling-stone shaped, 240–300 μ wide by 115–140 μ deep, sometimes conic Florence-flask shape and 175 μ deep. Ostiola 90–125 × 45 μ. Asci fusoid, acutely short-stipitate, mostly 4-spored, 36–40 × 6–9 μ. Sporidia hyaline, naviculate, 1-septate, 16–19 × 3–3½ μ.

Parasitic on leaves of *Alnus rhombifolia*, Riverside, Calif., Aug. 12, 1924. E. Bartholomew: *8896*. On *Alnus rubra*, Santa Cruz, Calif. Mary S. Clemens. (D: *5658*.)

Sydowia dothideoides Dearn. & Barth. sp. nov.

Perithecia cespitose or thickly scattered, rugose-membranaceous, developed upon or in a subcuticular, dematiaceous subiculum, erumpent, pseudostromata *Dothidea*-like encinctured by the ruptured cuticle, epapillate, sometimes gaping, .5–.75 mm. Asci cylindric or clavate-fusoid, narrowed apically, polysporous, aparaphysate, 90–160 × 15–20 μ. Sporidia hyaline, mostly irregularly 2-ranked, clavate-subfusoid with rounded ends, 3-

septate, occasionally a lengthwise septum in one of the cells and sometimes an additional cross-septum, strongly constricted, 16–23 μ long, the upper part 4½–9 μ wide, the lower part 3–6 μ wide.

On dead branchlets of *Populus tremuloides*, Jenny Lake, Wyo., July 14, 1924. E. Bartholomew: *8792*. (D: *5710*.)

Its apparently conidial stage is more abundant in the same collection. The pycnidia are smaller than the perithecia but otherwise similar; the conidia are hyaline, subelliptic, 7–10 × 3½–5½ μ. There are also dendryphioid conidia on the subiculum.

A scanty collection of what seems to be this species was also made on *Prunus melanocarpa* in the same locality, July 11, 1924. E. Bartholomew: *8782*.

CUCURBITARIA FRAXINI Ellis & Ev. Proc. Nat. Acad. Phila. 1890, 240:—In an excellent study of the genus *Cucurbitaria* by D. S. Welch, Mycologia **18**: 51–86, *C. Fraxini* Ellis & Ev. is placed in the doubtful list with the remark, p. 66, that the type specimen "furnishes little evidence of what the fungus might have been" and that Griffith's W. A. F. *198* on *Fraxinus* "is clearly not a *Cucurbitaria*." The type was sent to Mr. J. B. Ellis (I have the cotype) with several other collections on *Fraxinus* and erroneously labeled on that host instead of *Acer saccharinum*. A communicated correction of the error seems to have been overlooked. "*Fraxinus*" in the last line of page 239 in Ellis & Ev. N. Am. Pyrenom. should read "*Acer*" and in the legend of plate 26, ibid., "ash" should be "maple." Fig. 3 of the same plate should show a subiculous stroma under the perithecia—a feature that is quite distinct in the cotype. I am of opinion that the fungus is a form of *Cucurbitaria elongata* (Fries).

A specimen in good fruit on *Prunus melanocarpa*, E. Bartholomew, *6042*, adds a species to the list of hosts of *C. elongata*.

Cucurbitaria naucosa (Fries) Fuckel, Symb. Myc. 173:—Ellis & Ev. (N. Am. Pyrenom. 240) throw doubt on the American record of this species and D. S. Welch, l.c. 77, places it in the list of excluded species. My copy of Ellis & Ev. N. Am. Fungi *3356*, labeled *Dothiorella minor* Ellis & Ev., and related material, No. *491955* of the Field Museum, Chicago, bear, besides the species named on the label, fertile stromata agreeing with the description

in N. Am. Pyrenom. 240. The same material might very well pass for *Curreya excavata* (Cooke & Ellis), N. Am. Pyrenom., p. 618, which Thiess. & Syd. would call *Dictyodothis excavata* (Cooke & Ellis), Ann. Myc. **13**: 319.

Cucurbitaria Ceanothi Dearn. & House in the type material shows single as well as cespitose perithecia. The separate perithecia at least have the characters of *Pleospora*—**P. Ceanothi** (Dearn. & House) comb. nov.—as pointed out by D. S. Welch, l.c. 80.

Bagnisiopsis Eucalypti Dearn. & Barth. sp. nov.

Stromata subcircular to elongate, seated in the phloem, edges marked by the broken, slightly upturned epidermis, thinly covered by a somewhat friable remnant of the bark through which the ostiola of the loculi are barely visible, .5 to .75 mm. wide by .75 to 2 mm. long, or by confluence 3 to 5 mm. long. Loculi 60–280 μ in diameter, white-fleshed, in rather crowded groups or ranks. Asci cylindric-clavate, short-stipitate, paraphysate, 75–120 $\times$ 15–18 μ. Sporidia 2-ranked above, sometimes obliquely uniseriate, hyaline, continuous, grumous or guttate, elliptic or oftener obovoid, 17–23 $\times$ 9–11 μ.

In the bark of dead twigs of *Eucalyptus* sp., San Francisco, Cal., Aug. 2, 1924. E. Bartholomew: *8859*. (D: *5725*.)

Phragmodothidea Dearn. & Barth. gen. nov.

Stromata dothideous, asci paraphysate, ascospores phragmosporous. Differs from *Phragmodothella* Thiess. & Syd. only by being paraphysate and having more than 3 septa in the ascospore.

Phragmodothidea Eucalypti Dearn. & Barth. sp. nov.

Stromata black, rugose, circular or narrow-elongate and usually in linear series, seated in the dermis, rupturing the epidermis stellately or by a rift sometimes over the whole length but often only over the perforate mouths of the loculi, 100–300 μ wide, 55–90 μ deep. Loculi globose or flattish, one to ten in a stroma, often in a single row, white fleshed, 55–150 μ in diam. Asci 60–75 μ long, of various shapes, usually enlarged proximally, often bearing all the sporidia in the lower half and there 20–24 μ across or merely obclavate, 105 $\times$ 9–11 μ. Paraphyses linear, septate, seldom branched, longer than the asci. Sporidia hyaline, 6-celled when mature, elliptic-oblong, 17–24 μ long, end cells 3½–4½ μ wide, others 5½–7 μ wide.

In the bark of dead stems of *Eucalyptus* sp., San Francisco, Cal., Aug. 2, 1924. E. Bartholomew: *8859*. (D: *5724*.)

PHYLLACHORA DIPLOCARPA Ellis & Ev. Bull. Torrey Club **24**: 292. 1897.

Homostegia diplocarpa Ellis & Ev. Bull. Torrey Club **24**: 135. 1897.

On *Distichlis spicata*, S. Dakota, Sept. 1923. E. Barth.: *8437*.

Thiessen & Sydow, using Ellis & Ev. N. Am. Fungi *3439*, which in my copy is immature, transferred *P. diplocarpa* Ellis & Ev. to *Endodothella Tracyi* (Ellis & Ev.) Thiess. & Syd. Ann. Myc. **13**: 583. Ellis and Everhart state that they saw only stylospores. Material in both Fungi Columb. *4745*, Calif., and Bartholomew *8437* show not only the 3-septate stylospores ascribed to the species by the authors but also asci, 48–60 × 12 μ and continuous, hyaline sporidia 12 × 6 μ. *Phyllachora Tracyi* Ellis & Ev. Jour. Myc. **4**: 63 has 1-septate sporidia—the mark of *Endodothella*. I failed to find any septate sporidia in the apparently mature material mentioned above.

Oligostroma (*Phyllachora*) **acicola** sp. nov.

Stromata seated in the mesophyll, appearing as short, dark-gray lines under the cuticle, largest reaching 1 × .3 mm. Perithecial cells 1–10 in a stroma, mostly in a single row, sometimes in 2, rarely in 3 rows, depressed-globose, 65–80 μ in diameter. Asci aparaphysate, 30 × 6–9 μ, usually narrowed towards the top. Sporidia hyaline, obliquely uniseriate or biseriate near the base of the asci, uniseptate, 8–10 × 3 μ.

On withered needles of *Pinus palustris* associated with *Cryptosporium acicolum* Thum., Silver Springs, Fla., Feb. 27, 1919. G. G. Hedgcock: *32146*. Also G. G. H.: *17529*, *17627*, *20827*, *25156*, *32168*. (D: *5831*.)

Dichaena Pyri Dearn. & Barth. sp. nov.

(?) Stromata blackberry-like warts, circular-pulvinate, 1–3 mm., or transversely elongate up to 6 × 3 mm., 1½–2 mm. high, covered with perithecia, developing in the bark tissue, the core at base wood-colored, fine celled pseudoprosenchyma, opening towards the top before maturity into irregular cavities lined with filiform conidiophores, 10–25 μ long, bearing sperm-like conidia,

2½–3½ × ½ μ, forming, if not washed off, amber beads on the surface. Perithecia nearly black, subglobose or longer, irregular or confluent, areolate, coriaceo-membranous, 15–100 crowded over the stroma, .27–.45 mm. across, .4–.5 mm. high, opening rounded or somewhat elongate. Asci cylindrical, shortly pedicellate, 62–90 × 8–11 μ, 8-spored; paraphyses simple or branched longer than the asci. Sporidia hyaline, elliptic, grumous, 1- to 3- sometimes 5-septate, somewhat constricted in the middle, 10–16 × 6½–9 μ.

On bark round the base of dying apple tree, *Pyrus Malus*, Stockton, Kan., Oct. 1923. E. Bartholomew: *8587*. (D: *5427*.)

DEUTEROMYCETES

Phyllosticta Dracocephali Dearn. & Bisby, sp. nov.

Spots circular at first up to 3 mm. in diameter, then spreading and taking shape from the veinlet enclosures, the point of inception indicated by a white, circular spot 1 mm. in diameter, general color dull brown, somewhat paler beneath, often margined by a narrow, darker band. Pycnidia scattered, numerous, innate, globose, visible as dark specks under the upper cuticle, thin-walled, 120–130 μ deep. Conidia hyaline, densely granular, abundant, oblong with rounded ends, 7–13 × 3½–4½ μ.

On living leaves of *Dracocephalum parviflorum*, Winnipeg, Man., June 19, 1925. G. R. Bisby & I. L. Conners: *2014*. (D: *5867*.)

Phoma Physopellae Dearn. & Barth. sp. nov.

Uredinicolous, externally like *Darluca Filum* (Biv.), one to several botuliform to globose pycnidia, 60–100 × 60–90 μ, on a uredosorus; walls thin, parenchymatic; stomata conspicuous, 10–20 μ high, 15–18 μ wide. Conidia hyaline, continuous, 9–13 × 2.75–3 μ.

Parasitic on *Physopella ficina* on *Ficus aurea*, Cocoanut Grove, Fla., Feb. 28, 1924. L. W. Nuttall: *32*. (D: *5702*.)

Phomopsis Hibisci sp. nov.

Pycnidia erumpent but covered, seated deep in the cortex, scattered, .5–.6 mm.; wall very thin. Conidia hyaline, continuous, of two forms—hamate, 20–30 × 1 μ, and ellipsoid, 5–9 × 2.75 μ; conidiophores 15–30 × 1 μ.

On dead stems of *Hibiscus syriacus*, Orient, N. Y., May 28, 1923. R. Latham: *1717*. (D: *5451*.) This form seems to be somewhat like *Phoma ophites* Sacc.

Sclerotiopsis testudinea sp. nov.

Pycnidia scattered, long axis always parallel to the fibers of the stem, dark-brown, shining, testudinate, .5–1 × .3–.5 mm. Conidia hyaline, slightly curved, acute at each end, 5–8 × 1.75–2.25 μ; on simple and fasciculate-branched conidiophores, 10–30 × 1½–2 μ.

On dead stems of *Paeonia*, Orient, N. Y., Nov. 1919. R. Latham: *4012*. (D: *4442*.)

For an illuminating discussion of *S. concava* by Shear and Dodge with illustrations see Mycologia **13**: 147–9. *S. testudinea* differs externally, but only slightly in the pycnidial contents.

Dothiorella scopulina Dearn. & Barth. sp. nov.

Stromata thickly scattered, encinctured by the ruptured cuticle, disk gray at first, black when mature, .5–1.25 × .5–.75 mm. Loculi one to three or four in a stroma, .25–.5 mm. in diameter, 85–300 μ high, minutely papillate. Conidia hyaline, oblong with round ends, 10–14 × 4–5 μ, or elliptic, 7–10 × 4–6 μ.

On dead branchlets of *Sorbus scopulina*, Jackson Lake, Wyo., July 9, 1924. E. Bartholomew: *8773*. (D: *5709*.)

VALSELLA LASCHII Nits.

On dead branches of *Prunus Besseyi* (cult.), Agr. Coll., Winnipeg, Man., Sept. 1925. G. R. Bisby: *2197, 2198*. (D: *5911*.)

Both the *Valsella* and a *Cytospora* are on the same branches—the latter probably the undescribed conidial stage of the *Valsella*. Externally the stromata are indistinguishable. The conidia, 4–6 × 1 μ, on fasciculate, branched conidiophores, are developed in labyrinthine cells and escape by a central pore hardly visible in the middle of the white disk at the top of the stroma.

Ceuthospora Populi Dearn. & Overholts, sp. nov.

Stromata scattered, seated in the inner bark, raising the cuticle into subhemispheric pustules and rupturing it, often throwing it off, circular, ½–1 mm., to oblong, 2 × 1–1¼ mm., gray to brown-gray, one or sometimes two or three flat or papillate stomata. Loculi 2–6 in a stroma, more or less labyrinthine. Conidia hyaline, elliptic or oblong with rounded ends, 8–12 × 3–4 μ. Conidiophores 2–2½ μ thick in a layer 15–25 μ deep.

On dead twigs of *Populus grandidentata*, State College, Pa., Nov. 1, 1924. L. O. Overholts: *9460*. (D: *5674*.)

Ceuthospora is often accompanied with related single-celled pycnidia (*Phoma*). There are some such pycnidia on this material which at first contain similar spores, 9 × 3 μ, but they become 1-septate and colored (*Microdiplodia*).

Sphaeropsis amplispora Dearn. & Barth. sp. nov.

Pycnidia evenly scattered, pustulate, not erumpent but perforate and spore-inquinant, .5–.75 mm. in diam.; wall 15–30 μ thick. Conidia brown, nucleate, subglobose, elliptic, or ovate, large, 24–36 × 14–18 μ; on mostly clavate conidiophores, 15–40 × 5–7 μ.

On dead branches of *Acer saccharinum* L., Blue Rapids, Kan., Nov. 1898. E. Bartholomew: *2529*. (D: *5906*.)

Externally much like species of *Steganosporium* on this host.

Sphaeropsis Cercidis Dearn. & Barth. sp. nov.

Pycnidia thickly scattered or seriate, cortical, raising the bark into pustules .5 mm. in diameter. Conidia oblong-elliptic, of uniform content, 18–22 × 9–10 μ; conidiophores 8–17 μ.

On dead twigs of *Cercis canadensis*, Stockton, Kan., Oct. 1923. E. Bartholomew: *8577*. (D: *5429*.)

Sphaeropsis Lycii Dearn. & Barth. sp. nov.

Pycnidia scattered, 6–8 per sq. mm., minute, 150–190 μ, seated in the cortex, slightly erumpent. Conidia dark-brown, oblong-elliptic or pyriform, 14–18 × 9 μ. Conidiophores about 25 μ.

On dead branches of *Lycium vulgare*, St. Genevieve, Mo., Oct. 2, 1923. E. Bartholomew: *8573*. (D: *5428*.)

Haplosporella amygdalina Dearn. & Barth. sp. nov.

Stromata erumpent, embraced by the ruptured cuticle, black, thickly scattered, .25–.45 mm., containing 1–5 pycnidia. Pycnidia obtusely papillate, .125 mm., the stoma only or part of the body up to one third rising above the level of the stroma. Conidia pale brown, content uniform, oblong with rounded ends, enucleate, 15–19 × 7–8 μ, on long narrow conidiophores, 8–15 × 1 μ.

On *Amygdalus Persica*, Stockton, Kan., Apr. 24, 1924. E. Bartholomew: *8673*. (D: *5568*.)

Haplosporella Lathami sp. nov.

Stromata thickly scattered, erumpent, encinctured and partly covered by the raised bark, dull black, nearly flat, sometimes showing the rounded domes of the pycnidia, .3–1.5 mm. Pycnidia 2–8 in a stroma, 150–180 μ in diameter, partitions 30–85 μ thick. Conidia brown, continuous, enucleate, oblong with rounded ends, 15–21 $\times$ 6–9 μ; conidiophorous layer about 22 μ deep.

On dead twigs of *Myrica carolinensis*, Mattituck, N. Y., Jan. 1924. Roy Latham: *1585*. (D: *5433*.)

Diplodiopsis Robiniae Dearn. & Barth. sp. nov.

Mature stromata erumpent, black, .5–1.8 $\times$ .3–.5 mm., seriate in lines or scattered. Pycnidia in well developed stromata 85 μ in diameter, marked by their hemispheric top which terminates in a shining black ostiolum, 60–85 μ high and 30–35 μ wide, 2–17 in a stroma. Conidia hyaline at first, becoming pale-brown and 1-septate, exceptionally 2-septate, oblong-elliptic, subacute at the proximal end and rounded at the other, 9–20 $\times$ 5–8 μ; conidiophores 20–30 μ.

On dead branches of *Robinia pseudacacia*, Stockton, Kan., Nov. 28, 1923. E. Bartholomew: *8610*. (D: *5432*.)

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(To be Concluded)